NTP REPORT

ON THE

TOXICOLOGY STUDIES OF

TRIMETHYLOLPROPANE TRIACRYLATE

(Technical Grade)
(CAS NO. 15625-89-5)

IN F344/N RATS, B6C3F$_1$ MICE, AND GENETICALLY MODIFIED (FVB Tg.AC HEMIZYGOUS) MICE

(DERMAL STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

October 2005

NTP GMM 3

NIH Publication No. 06-4450

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the studies were subjected to retrospective quality assurance audits before being presented for public review.

The studies described in this Report series were designed and conducted to characterize the toxicologic potential, including carcinogenic activity, of selected agents in laboratory animals that have been genetically modified. These genetic modifications may involve inactivation of selected tumor suppressor functions or activation of oncogenes that are commonly observed in human cancers. This may result in a rapid onset of cancer in the genetically modified animal when exposure is to agents that act directly or indirectly on the affected pathway. An absence of a carcinogenic response may reflect either an absence of carcinogenic potential of the agent or that the selected model does not harbor the appropriate genetic modification to reduce tumor latency and allow detection of carcinogenic activity under the conditions of these subchronic studies. Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection per se is not an indicator of a chemical's carcinogenic potential.

Details about ongoing and completed NTP studies, abstracts of all NTP Reports, and full versions of the completed reports are available at the NTP's World Wide Web site: http://ntp.niehs.nih.gov. In addition, printed copies of these reports are available from NTP as supplies last by contacting (919) 541-3419.

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CONTENTS

SUMMARY

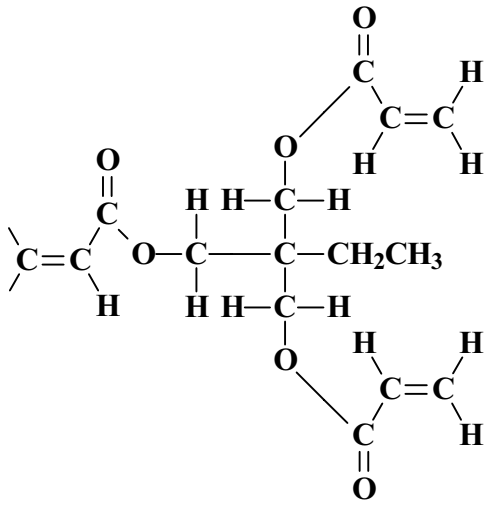
Background
Trimethylolpropane triacrylate is used in a variety of photoreactive products including inks and coatings. People are exposed to trimethylolpropane triacrylate mainly by skin contact. We used a genetically modified strain of mouse with sensitive skin to test if trimethylolpropane triacrylate might cause skin cancer.

Methods
We painted solutions of trimethylolpropane triacrylate dissolved in acetone on the backs of male and female Tg.AC mice five times per week for 6 months. The daily doses were 0.75, 1.5, 3, 6, or 12 milligrams of trimethylolpropane triacrylate per kilogram body weight. Animals painted with acetone alone served as the control groups. Tissues from 15 sites were examined for every animal.

Results
Almost all the mice receiving daily doses of 3, 6, or 12 mg/kg developed a variety of precancerous or cancerous skin lesions at the site of chemical application. These included epithelial hyperplasia and squamous cell papilloma. Female mice also had increased incidences of forestomach papilloma.

Conclusions
We conclude that trimethylolpropane triacrylate caused skin papillomas in the genetically modified mouse model used in these studies. Development of forestomach papillomas in female mice may also have been related to trimethylolpropane triacrylate.

ABSTRACT



TRIMETHYLOLPROPANE TRIACRYLATE

CAS No. 15625-89-5

Chemical Formula: $C_{15}H_{20}O_6$ Molecular Weight: 296.3

Synonyms: Acrylic acid, 2-ethyl-2-(((1-oxo-2-propenyl)oxy)methyl)-1,3-propanediol triester; A-TMPT; 2-ethyl-2-(hydroxymethyl)-1,3-propanediol triacrylate; 2-ethyl-2-(((2-oxo-2-propenyl)oxy)methyl)-1,3-propanediyl ester; 2-propenoic acid, 2-ethyl-2-(((1-oxo-2-propenyl)oxy)methyl)-1,3-propanediol ester; TMPTA; 1,1,1-trimethylolpropane triacrylate

Trade names: Aronix M 3090, Monosizer TD 1500A, NK Ester A-TMPT, SARTOMER SR 351, Setalux UV 2241, SR 351, Viscoat

Trimethylolpropane triacrylate is a multifunctional monomer with a wide range of industrial applications. It is used in the production of ultraviolet-curable inks, electron beam irradiation-curable coatings, and polymers and resins; as a component of photopolymer and flexographic printing plates and photoresists; and as an ingredient in acrylic glues and anaerobic sealants. The chemical is also used in paper and wood impregnates, wire and cable extrusion, polymer-impregnated concrete, and polymer concrete structural composites. Trimethylolpropane triacrylate was nominated by the National Cancer Institute for testing due to its high production volume and use, its potential for consumer exposure, and a lack of adequate testing of the chemical. Male and female F344/N rats and B6C3F$_1$ mice were administered technical grade trimethylolpropane triacrylate (it is reactive and therefore not available as pure trimethylolpropane triacrylate) in acetone dermally for 2 weeks or 3 months. Male and female Tg.AC hemizygous mice were administered technical grade trimethyl-olpropane triacrylate in acetone for 6 months. Genetic toxicology studies were conducted in B6C3F$_1$ and Tg.AC hemizygous mouse peripheral blood erythrocytes.

2-Week Study in Rats

Groups of five male and five female F344/N rats were administered 0, 12.5, 25, 50, 100, or 200 mg trimethyl-olpropane triacrylate/kg body weight in acetone 5 days per week for 16 days. All rats survived to the end of the study, and mean body weights of dosed groups were similar to those of the vehicle controls. Dosed rats had irritation at the site of application; this clinical finding was

most commonly seen in rats administered 50 mg/kg or greater. Male and female rats had epidermal hyperplasia, hyperkeratosis, sebaceous gland hyperplasia, inflammation of the epidermis and dermis, ulceration, epidermal degeneration, and parakeratosis at the site of application.

2-Week Study in B6C3F$_1$ Mice

Groups of five male and five female B6C3F$_1$ mice were administered 0, 12.5, 25, 50, 100, or 200 mg trimethylolpropane triacrylate/kg body weight in acetone 5 days per week for 16 days. All mice survived to the end of the study. The final mean body weight gain of 200 mg/kg males was less than that of the vehicle controls; 100 and 200 mg/kg females had significantly increased final mean body weights. Irritation at the site of application occurred in all dosed males, all 100 and 200 mg/kg females, and one 50 mg/kg female. Thymus weights of males administered 50 mg/kg or greater were significantly decreased. Dosed male and female mice had epidermal hyperplasia, hyperkeratosis, chronic active inflammation of the dermis, sebaceous gland hyperplasia, ulcer, epidermal degeneration, parakeratosis, and/or suppurative inflammation of the epidermis at the site of application. Atrophy of the thymus occurred in 100 and 200 mg/kg male mice.

3-Month Study in Rats

Groups of 10 male and 10 female F344/N rats were administered 0, 0.75, 1.5, 3, 6, or 12 mg trimethylolpropane triacrylate/kg body weight in acetone 5 days per week for 14 weeks. All rats survived to the end of the study, and mean body weights of dosed groups were similar to those of the vehicle controls. Irritation at the site of application was noted in five males and all females administered 12 mg/kg. Hematology results indicated that trimethylolpropane triacrylate at the doses selected induced a neutrophil count increase at 12 mg/kg that would be consistent with an inflammatory response related to the dermatitis observed histopathologically. Thymus weights of 12 mg/kg males and 0.75 and 12 mg/kg females were decreased. Incidences of epidermal hyperplasia, degeneration, and necrosis (females only); chronic active inflammation of the dermis, hyperkeratosis, and sebaceous gland hyperplasia were generally increased at the site of application in 1.5 mg/kg or greater males and in 3 mg/kg or greater females.

3-Month Study in B6C3F$_1$ Mice

Groups of 10 male and 10 female B6C3F$_1$ mice were administered 0, 0.75, 1.5, 3, 6, or 12 mg trimethylolpropane triacrylate/kg body weight in acetone 5 days per week for 14 weeks. All animals survived to the end of the study; mean body weights of dosed groups were similar to those of the vehicle controls. Irritation at the site of application occurred in male and female mice administered 12 mg/kg. Hematology results indicated that trimethylolpropane triacrylate induced a neutrophil count increase at 12 mg/kg that would be consistent with an inflammatory response related to the dermatitis observed histopathologically. Increased incidences of several nonneoplastic lesions occurred at the site of application in 3 mg/kg and greater males and females, including hyperplasia of the epidermis, hyperkeratosis, epidermal degeneration (except 3 mg/kg females) and necrosis, chronic active inflammation of the dermis, and sebaceous gland hyperplasia. Epidermal suppurative inflammation and necrosis and dermal fibrosis occurred in 12 mg/kg males and females.

6-Month Study in Tg.AC Hemizygous Mice

Groups of 15 male and 15 female Tg.AC hemizygous mice were administered 0, 0.75, 1.5, 3, 6, or 12 mg trimethylolpropane triacrylate/kg body weight in acetone 5 days per week for 28 weeks. Additional groups of 15 male and 15 female mice maintained as positive controls received dermal applications of 1.25 µg 12-*O*-tetradecanoylphorbol-13-acetate per 100 mL acetone 3 days per week for 28 weeks; the dosing volume was held constant at 100 µL. Survival and mean body weights of dosed groups were similar to those of the vehicle controls throughout the study. Treatment-related clinical findings included papillomas at the site of application in 3 mg/kg and greater males and 6 and 12 mg/kg females.

The heart weights of males and females administered 12 mg/kg and the kidney and lung weights of 12 mg/kg females were significantly increased. The lung weights of 6 and 12 mg/kg males and females were decreased.

Squamous cell neoplasms at the site of application were associated with dermal application of trimethylolpropane triacrylate. At 6 months, the incidences of squamous cell papilloma were significantly increased in 6

and 12 mg/kg males and females. One female in each of the 1.5, 6, and 12 mg/kg groups also had squamous cell carcinoma. The incidence of squamous cell papilloma of the forestomach in 12 mg/kg females was significantly greater than that in the vehicle control group.

Nonneoplastic skin lesions at the site of application in dosed mice included epidermal hyperplasia, hyperkeratosis, and chronic active inflammation. A hematopoietic disorder (myelodysplasia) also occurred in some 12 mg/kg males and females.

GENETIC TOXICOLOGY

No increase in the frequency of micronucleated erythrocytes was observed in peripheral blood samples from male or female $B6C3F_1$ mice treated with trimethylolpropane triacrylate by skin painting for 3 months. Similarly, no increase in micronucleus frequency was seen in male or female Tg.AC hemizygous mice administered trimethylolpropane triacrylate by skin painting for 6 months.

CONTACT HYPERSENSITIVITY STUDIES

Studies were conducted with female BALB/c mice to evaluate the potential for trimethylolpropane triacrylate to induce contact hypersensitization. In an irritancy study in which the chemical, in acetone, was applied to the ear, the maximal nonirritating and minimal irritating doses were 0.1% and 0.25% trimethylolpropane triacrylate. No significant differences in the percentage of ear swelling occurred between trimethylolpropane triacrylate-sensitized and -challenged mice and background controls at 24 or 48 hours after dosing. The local lymph node assay indicated no significant increase in lymph node cell proliferation in mice administered trimethylolpropane triacrylate compared to that in the vehicle controls. Testing for sensitizing potential using the mouse ear swelling test and local lymph node assay failed to indicate trimethylolpropane triacrylate as a potential contact sensitizer.

CONCLUSIONS

Male and female Tg.AC hemizygous mice dosed with trimethylolpropane triacrylate for 6 months had significantly increased incidences and multiplicity of papillomas of the skin at the site of dermal application. Treatment-related squamous cell carcinomas occurred at the site of application in dosed female mice. Increased incidences of forestomach squamous cell papilloma in female mice may have been related to chemical administration.

Increased incidences of minimal to moderate (mostly mild) hyperplasia of the epidermis, hyperkeratosis, and chronic active inflammation also occurred at the site of application. A hematopoietic disorder (myelodysplasia) also occurred in exposed male and female mice.

Summary of the 6-Month Toxicology and Genetic Toxicology Studies of Trimethylolpropane Triacrylate

	Male Tg.AC Hemizygous Mice	Female Tg.AC Hemizygous Mice
Doses in acetone by dermal application	Vehicle control, 0.75, 1.5, 3, 6, or 12 mg/kg	Vehicle control, 0.75, 1.5, 3, 6, or 12 mg/kg
Body weights	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group
Survival rates	14/15, 15/15, 12/15, 14/15, 13/15, 11/15	15/15, 14/15, 12/15, 14/15, 14/15, 12/15
Nonneoplastic effects	Skin (site of application): epidermal hyperplasia (0/15, 0/15, 0/15, 6/15, 14/15, 15/15); hyperkeratosis (0/15, 0/15, 1/15, 15/15, 14/15, 12/15); chronic active inflammation (0/15, 0/15, 1/15, 1/15, 9/15, 12/15) All organs: myelodysplasia (0/15, 0/15, 0/15, 0/15, 0/15, 2/15)	Skin (site of application): epidermal hyperplasia (0/15, 0/15, 1/15, 4/15, 15/15, 15/15); hyperkeratosis (0/15, 0/15, 1/15, 7/15, 14/15, 13/15); chronic active inflammation (0/15, 0/15, 0/15, 3/15, 14/15, 12/15) All organs: myelodysplasia (0/15, 0/15, 0/15, 0/15, 0/15, 2/15)
Neoplastic effects	Skin (site of application): squamous cell papilloma (0/15, 0/15, 0/15, 2/15, 12/15, 13/15)	Skin (site of application): squamous cell papilloma (0/15, 0/15, 0/15, 1/15, 11/15, 15/15); squamous cell carcinoma (0/15, 0/15, 1/15, 0/15, 1/15, 1/15)
Uncertain findings	None	Forestomach: squamous cell papilloma (4/15, 5/15, 4/15, 2/15, 5/15, 9/15)
Genetic toxicology		
Micronucleated erythrocytes		
Mouse peripheral blood *in vivo:*		
$B6C3F_1$	Negative in males and females	
Tg.AC hemizygous	Negative in males and females	

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Report on trimethylolpropane triacrylate on September 6, 2002, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On September 6, 2002, the draft Report on the toxicology and carcinogenesis studies of trimethylolpropane triacrylate received public review by the National Toxicology Program's Board of Scientific Counselor's Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. C.J. Portier, NIEHS, introduced the review by explaining that, as a result of consultation with the committee prior to the meeting, the original plan to evaluate the conclusions concerning possible carcinogenic hazards of the two multifunctional acrylates of trimethylolpropane triacrylate and pentaerythritol triacrylate had changed. The revised purpose of the review would be to evaluate the design and appropriate methods of analysis for these transgenic mouse studies. Further questions to be addressed were whether the Levels of Evidence of Carcinogenic Activity categories currently used for standard 2-year cancer bioassays would be applicable and what reporting format would be appropriate.

Dr. J.R. Bucher, NIEHS, outlined the agenda of presentations and described a variety of design and interpretation issues related to the use of genetically modified mouse models for cancer assessment. These included choice of animal model, group size, study duration, route of exposure, dose selection, use of positive or negative controls, extent of pathology evaluation, and appropriate statistical analysis method. Other fundamental questions were whether these should be considered carcinogenicity studies or promotion studies and what sort of interpretive conclusions could be drawn.

Dr. J.E. French, NIEHS, described the Tg.AC transgenic mouse model, the construction of the v-Ha-*ras* transgene, and the use of the model as a squamous epithelium reporter phenotype. Dr. D.B. Dunson, NIEHS, described a generalized Poisson system to analyze the incidence, multiplicity, and onset times for the skin papillomas that are the primary endpoint of the Tg.AC model.

Dr. R.S. Chhabra, NIEHS, described the study nomination and uses of the two chemicals, trimethylolpropane triacrylate and pentaerythritol triacrylate, the results of the traditional 2-week and 3-month toxicity studies, and the protocol and results for the 6-month transgenic mouse study of trimethylolpropane triacrylate. Effects observed were hyperplasia, hyperkerotosis, inflammation, and squamous cell papillomas of the skin at the site of chemical application in males and females, plus skin carcinomas in females, and myelodysplasia (a hematopoietic disorder) in males and females. In the standard mouse bioassay system the tumor response would have been judged "clear evidence of carcinogenic activity." Dr. J.R. Hailey, NIEHS, described the histologic characterization of the skin lesions observed and contrasted the more severe inflammatory responses to chemical exposure in the skin of $B6C3F_1$ mice to the milder inflammatory response in the Tg.AC mice.

Dr. Elwell, the first principal reviewer, agreed that the squamous cell neoplasms observed in the trimethylolpropane triacrylate study could be considered a positive response. He asked for more information on the response to the positive control (TPA) and expressed concern that the papilloma response was observed only at doses that also caused skin inflammation.

Dr. Storer, the second principal reviewer, suggested that a different form of conclusion other than "evidence of carcinogenic activity" would be more appropriate to describe results in the Tg.AC model. He also argued that it was unclear that the model would give equivalent responses as the classic skin promotion model. He inquired if a time sequence of histologic observations might help distinguish between two mechanisms for tumor formation, particularly in the forestomach: systemic exposure to inflammatory cytokines as a consequence of skin irritation or direct oral exposure to the chemical from grooming.

Dr. Piegorsch, the third prinicipal reviewer, felt the Dunson statistical model was reasonable, noting it may be specific for the Tg.AC system.

Dr. Chhabra noted that the systemic effect was seen only in one animal group, the high dose female group in the trimethylolpropane triacrylate study. Dr. French added that the dose regimen was determined operationally based on TPA doses that provided a robust response without being overtly toxic. In response to Dr. Storer's

question about time progression of tumorigenesis, Dr. Chhabra noted that the papillomas formed quickly, in a matter of a few weeks, and Dr. French added that the papillomas kept developing with chemical administration, so there was no acclimation or adaptation to exposure. Dr. Hailey said that the hematopoietic proliferation was thought to be associated with the myeloid rather than the erythroid component and thus more likely attributable to the inflammatory response rather than systemic exposure. Dr. Storer asked if one could infer that the *zeta*-globin promoter construct of the transgene was responsive to the inflammatory cytokines. Dr. French answered that, while that was a possibility, the proliferation more likely was a generalized response of the hematopoietic system.

Dr. Thrall said that use of complete blood count would have helped discern whether myelodysplasia, a preleukemic condition, or just an inflammatory response, occurred. Dr. Hailey agreed.

Dr. Walker inquired if there would be a qualitative difference in interpretation of response if some gene other that *ras* (for example, green fluorescent protein) were joined to and activated by the *zeta*-globin promoter. That is, whether cancer or some other gene expression was the endpoint of the model. Dr. French replied that there were two contexts for the expression of the gene in the Tg.AC model, that it was correctly turned on at day 12 of the embryogenesis, and that other regulatory control regions were also being brought into play.

Dr. Ho also questioned whether Tg.AC could be termed a cancer model and inquired if functional genomics or chromosomal characterizations had been done for the observed neoplasms. Dr. French replied that the primary focus had been on the downstream events for *ras* expression: *p53* mutation or inactivation. About 30% of the metaphase cells showed trisomy at chromosome 15, but changes in chromosome number did not seem a prerequisite for expression.

Dr. Vore asked about the relationship between inflammation or wounding and papilloma formation. Dr. French cited examples of studies where both effects were observed and other studies where either inflammation or papilloma formation occurred without the other. Dr. Chhabra added that one of the dose groups in the companion pentaerythritol triacrylate study was another such example.

Dr. Boekelheide asked if any difference in responses had been observed between sexes, and if the available surface changed once papillomas began forming. Dr. French noted that in a study of benzene the magnitude of response was greater in males. Whether that was due to a hormonal difference or because of different animal housing conditions was speculative. He also felt that any additional dosing after papillomas had begun forming was superfluous because the process was irreversible.

Dr. J. Van Miller, representing the American Chemistry Council (ACC) discussed the general class of chemicals known as specialty acrylates and methacrylates (SAMs), the use of monomeric forms of SAMs in producing cross-linked polymers, and a series of industry studies on two representative chemicals of this group (triethylene glycol diacrylate and the corresponding methacrylate). He noted that skin irritation is characteristic of SAMs, but they are not carcinogenic in the bioassays conducted by the ACC. He suggested that the skin tumors observed in the present Tg.AC studies may have been driven by irritation and urged that conclusions about carcinogenicity be withdrawn until the mechanism of papilloma formation was clarified. Dr. J. Allen, representing the ACC, also addressed the severe dermal toxicity in the trimethylolpropane triacrylate and pentaerythritol triacrylate studies and suggested that the observed tumors resulted from nonspecific skin toxicity.

Because of the general similarity of findings between the two acrylate studies, the panel agreed to forego a formal presentation on the companion pentaerythritol triacrylate study. Dr. Walker inquired about the seeming low purities of pentaerythritol triacrylate cited in the report. Dr. Chhabra explained that these were highly reactive materials and those measures were just of the monomer, whereas the technical grade material consisted of a mixture including oligomers and other monofunctional acrylates.

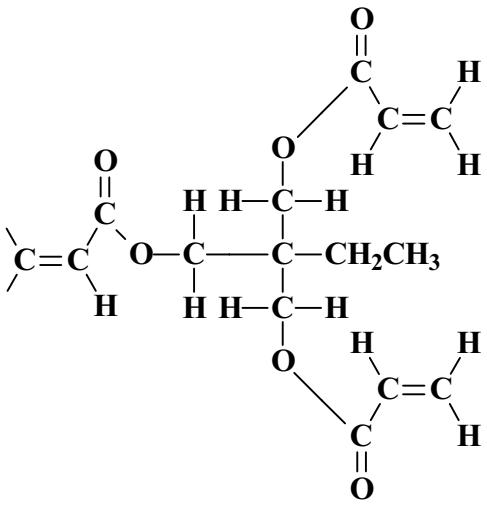
Dr. Drinkwater then turned the discussion to the general question of the use of transgenic models by the NTP, noting that suggestions have ranged from use as a preliminary screen to complete replacement of the conventional bioassay. Dr. Ho expressed optimism that the shorter time involved in the transgenic assays would enable rapid decisions about which tests would be most appropriate for a given chemical. Dr. Storer

diffe rentiated between reporter models, such as the Tg.AC model, and oncogene or tumor suppressor gene models such as *p53* or *ras*-H2. He felt the latter might merit conclusions about carcinogenicity, whereas systems such as the Tg.AC model would more appropriately be used as part of larger summaries of a collection of studies. Dr. Piegorsch noted that in an NTP evaluation of various strategies for identifying carcinogens, the strongest concordance came from a combination of data from *p53* and traditional rat bioassays and genotoxicity. Responding to Dr. Drinkwater's suggestion, Dr. Storer agreed the Tg.AC studies might fit better in a different type of report than the Technical Report series.

Drs. Walker and Elwell inquired about how positive and negative results from transgenic studies would be used in decisions about whether to perform additional testing. Dr. Klaunig concurred with the notion of using the transgenic models in a triage approach for testing and also emphasized the need to understand the mechanism for tumor formation in such models. Regarding the type of interpretive conclusion that can be drawn from transgenic models, Dr. Roberts noted that many of the transgenic models might not be predictive for carcinogenicity *per se*. Dr. Storer felt that while the *p53* or *ras*-H2 models more closely approximated the normal tumorigenic processes, the Tg.AC model was more questionable in that regard. Dr. Walker concurred.

No vote was taken on the conclusion statements in the draft reports.

INTRODUCTION



TRIMETHYLOLPROPANE TRIACRYLATE

CAS No. 15625-89-5

Chemical Formula: $C_{15}H_{20}O_6$ Molecular Weight: 296.3

Synonyms: Acrylic acid, 2-ethyl-2-(((1-oxo-2-propenyl)oxy)methyl)-1,3-propanediol triester; A-TMPT; 2-ethyl-2-(hydroxymethyl)-1,3-propanediol triacrylate; 2-ethyl-2-(((2-oxo-2-propenyl)oxy)methyl)-1,3-propanediyl ester; 2-propenoic acid, 2-ethyl-2-(((1-oxo-2-propenyl)oxy)methyl)-1,3-propanediol ester; TMPTA; 1,1,1-trimethylolpropane triacrylate

Trade names: Aronix M 3090, Monosizer TD 1500A, NK Ester A-TMPT, SARTOMER SR 351, Setalux UV 2241, SR 351, Viscoat

Chemical and Physical Properties

Trimethylolpropane triacrylate is a viscous, colorless liquid with an acrylic or pungent odor and a boiling point greater than 200° C at 1 mm Hg (Lenga, 1988; Alfa, 1990). At 25° C, it has a specific gravity of 1.1084 and a vapor pressure of less than 1.0 mm Hg (AIHA, 1981; ARCO, 1989); the refractive index is 1.4736 at 20° C (Lenga, 1988). It is insoluble in water and is hygroscopic, light sensitive, and incompatible with strong oxidizing agents, acids, and bases. Trimethylolpropane triacrylate may undergo spontaneous polymerization when exposed to direct sunlight and heat but may be stabilized with the monomethyl ester of hydroquinone (AIHA, 1981; Lenga, 1988). Trimethylolpropane triacrylate is combustible, with a flashpoint of greater than 110° C (Lenga, 1988). The chemical is reactive and is therefore not available as pure trimethylolpropane triacrylate.

Production, Use, and Human Exposure

Trimethylolpropane triacrylate is manufactured from trimethylolpropane (*Kirk-Othmer*, 1978); acrylic acid is a known impurity in the technical-grade compound (Celanese, 1982). The U.S. Environmental Protection Agency's Toxic Substances Control Act Inventory for 1983 indicated that 120,000 to 1.2 million pounds of trimethylolpropane triacrylate were produced in the

United States by companies providing annual production volumes; no information on imported amounts was available (USEPA, 1990).

Trimethylolpropane triacrylate 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, electron beam irradiation-curable coatings, and polymers and resins; as a component of photopolymer and flexographic printing plates and photoresists; and as an ingredient in acrylic glues, adhesives, and anaerobic sealants. It is used in colloidal dispersions for industrial baked coatings, waterborne and solvent-based alkyds, and vinyl/acrylic nonwoven binders. Additionally, trimethylolpropane triacrylate is used in paper and wood impregnates, wire and cable extrusion, polymer-impregnated concrete, and polymer concrete structural composites (Celanese, 1982; Björkner, 1984; Dearfield *et al.*, 1989; Radak, 1990).

Workers involved in the manufacturing, processing, product handling, and application of trimethylolpropane triacrylate are at risk of exposure (AIHA, 1981). Surveys by the National Institute for Occupational Safety and Health (1990) indicated that approximately 4,180 workers were exposed to trimethylolpropane triacrylate between 1981 and 1983. The American Industrial Hygiene Association (1981) established a workplace environmental exposure level (8-hour time-weighted average) of 1 mg/m^3 trimethylolpropane triacrylate; no other exposure regulations or recommendations have been established. A potential exists for widespread exposure of consumers through the use of trimethylolpropane triacrylate in products such as latex paints and floor polishes (Dearfield *et al*., 1989).

Absorption, Distribution, and Excretion

In male F344/N rats, 18.7% of a single dermal dose of 130 mg/kg [^{14}C]-trimethylolpropane triacrylate was absorbed and an average of 76% of the administered radiolabel was recovered unabsorbed 72 hours after dosing (Appendix L). In rats administered a 24-hour preexposure dose of nonradiolabeled trimethylolpropane triacrylate, 25% of a subsequent 151 mg/kg radiolabeled dose was absorbed and 65% was recovered unabsorbed from the dose site 72 hours after dosing. The absorption of dermally administered trimethylolpropane triacrylate was inversely related to dose; a total of 18.7% of the 130 mg/kg dose was absorbed, while 32.7% of the 15.2 mg/kg and 55.1% of the 1.7 mg/kg dose were absorbed. In mass terms, approximately five times more trimethylolpropane triacrylate was absorbed as the dose concentration increased by one order of magnitude.

An average of less than 5% of the dose was recovered in the excreta of rats 72 hours after dermal application of 130 mg/kg, compared to an average of approximately 19% of the 15.2 mg/kg dose and 45% of the 1.7 mg/kg dose (Appendix L). Very little radioactivity was associated with most of the tissues 72 hours after exposure; however, the kidney had elevated tissue:blood ratios at each dose concentration.

In male B6C3F$_1$ mice, a total of 75% of dermally applied [^{14}C]-trimethylolpropane triacrylate was absorbed 72 hours after a single 1.2 mg/kg dose (Appendix L). A much larger percentage of the applied radioactivity remained at the site of application in mice than in rats (31% in mice, 9% in rats). The collected tissues and residual carcass contained less than 2% of the administered dose. Approximately 21% of the dose remained unabsorbed, and approximately 42% was excreted in the urine, feces, and exhaled carbon dioxide after 72 hours. Very little radiolabel was associated with most of the tissues 72 hours after dosing; the non-application site skin, however, had an elevated tissue:blood ratio.

During the 72 hours following an intravenous bolus dose of 9.4 mg/kg [^{14}C]-trimethylolpropane triacrylate to rats, a total of 77.4% of the radiolabel was excreted in the urine, feces, and exhaled carbon dioxide, with urine and exhaled carbon dioxide accounting for the largest percentages (Appendix L). Total radiolabel (but not parent trimethylolpropane triacrylate) was measured in the blood, and only slight changes in radiolabel concentrations were observed in blood after 1 hour. Among the tissues collected 72 hours after dosing, the highest radiolabel concentration was in the blood, and the average total recovery of radiolabel was 90% during the 72 hours after the intravenous dose.

To determine if the elevated kidney:blood ratios following dermal dosing were due to covalent binding of radiolabeled compounds to tissue macromolecules, the binding of ^{14}C to kidney samples from dermal and intravenous studies in rats was determined (Appendix L). The high kidney:blood ratios in dermally treated rats were not due to covalent binding of the radiolabeled

compounds to kidney protein, but were probably associated with urine. In contrast, in the intravenous bolus study, the systemically available trimethylolpropane triacrylate resulted in covalent binding to macromolecules associated with the kidney.

TOXICITY

Experimental Animals

Acute oral LD_{50} values reported for trimethylolpropane triacrylate in rats were 3.84 to 7.01 mL trimethylolpropane triacrylate/kg body weight (Carpenter *et al*., 1974) or 5,000 mg/kg (Andrews and Clary, 1986). Dermal LD_{50} values for rabbits were 200 to 2,000 mg/kg (Andrews and Clary, 1986), 3.89 to 10.04 mL/kg (Carpenter *et al*., 1974), or 5,170 mg/kg for a 24-hour exposure period (AIHA, 1981).

In a series of studies conducted by Celanese Corporation, Inc. (Andrews and Clary, 1986), trimethylolpropane triacrylate was administered undiluted or in acetone or mineral oil to the interscapular region of male C3H/HeJ mice. All five mice administered 50 mg undiluted trimethylolpropane triacrylate died 1 day after treatment; clinical findings included lethargy, inactivity, and salivation shortly after application. A group of three mice administered 50 mg of a 10% solution in acetone twice per week for 2 weeks developed epilated, crusty, severely burned skin. Very slight crusting of the skin was observed in mice treated with 50 mg of a 5% solution in mineral oil twice per week for 5 weeks.

Immunized, outbred, male and female Hartley guinea pigs (number per group not reported) received daily applications of 0.2 mL of a solution of 0.1%, 0.25%, or 0.5% trimethylolpropane triacrylate in acetone:olive oil (4:1) for 7 days (Parker and Turk, 1983). Skin reactions were graded on a scale of 0 (no reaction) to 3 (severe reaction). The guinea pigs developed mild to moderate skin sensitization.

In a sensitization study by Nethercott *et al.* (1983), groups of 10 female albino Hartley/Dalkin guinea pigs were induced and then challenged with trimethylolpropane triacrylate. Three intradermal injections were administered to each shoulder: 0.1 mL of a 0.5% or 10% solution of trimethylolpropane triacrylate in propylene glycol; 0.05 mL Freund's Complete Adjuvant (FCA) and 0.05 mL of a 0.5% or 10% solution of trimethylolpropane triacrylate in propylene glycol; and 0.1 mL FCA. After 1 week, 0.5% or 10% trimethylolpropane triacrylate in petrolatum was applied to the animals' shaved shoulders, which were then wrapped for 48 hours. The animals were challenged 2 weeks after the topical exposure with skin patches of nonirritant concentrations of trimethylolpropane triacrylate for 24 hours. Four guinea pigs that were administered 0.5% trimethylolpropane triacrylate and 10 of 20 administered the 10% solution became sensitized; the intradermal sensitivity concentration for 50% of the animals was determined to be 5.4%.

In a maximization test to determine skin sensitivity and cross-sensitivity reactions, groups of 15 female albino Dunkin/Hartley guinea pigs were sensitized topically with 25% solutions of commercial-grade pentaerythritol tri- or tetraacrylate in petrolatum and then challenged with two applications on the flank, 1 week apart, of 0.015 g pentaerythritol tri- or tetraacrylate (commercial grade and purified) or trimethylolpropane triacrylate in petrolatum (Björkner, 1984). A booster of the sensitizing chemical was administered intradermally on the neck 48 hours after the first challenge. Of the 10 animals that became sensitized to commercial-grade pentaerythritol triacrylate, seven also reacted to trimethylolpropane triacrylate. Only one guinea pig became sensitized to commercial-grade pentaerythritol tetraacrylate; this animal also reacted to trimethylolpropane triacrylate. These results indicated that pentaerythritol triacrylate was the more potent sensitizer and that guinea pigs sensitized to pentaerythritol triacrylate may cross-react to trimethylolpropane triacrylate.

In a maximization test of acrylates and methacrylate esters, outbred female SSc:AL guinea pigs were induced with three 2 × 50 µL intradermal injections, including one of FCA in sterile water and one each of a test compound (methyl methacrylate, ethyleneglycol dimethacrylate, triethyleneglycol dimethacrylate, or trimethylolpropane trimethylacrylate) in soybean oil and in a mixture of emulsified FCA and water (Clemmensen, 1984). On day 7, approximately 250 mg of 10% sodium lauryl sulfate in petrolatum was applied to the neck and left uncovered for 24 hours. On day 8, the test compound or petrolatum (400 µL) was applied to a filter paper patch which was applied to the flank and left in place for 48 hours. On day 21, the guinea pigs were challenged with up to six patches containing 25 µL of the sensitizing compound; 2-hydroxy-methacrylate; 1,6-hexane diolodiacrylate; pentaerythritol triacrylate;

or trimethylolpropane triacrylate. Sensitization determinations were made at 48 and 72 hours. The treatment was repeated on the opposite flank of each animal after 35 days. Positive skin sensitization reactions occurred in 14 of 19 guinea pigs induced with trimethylolpropane trimethylacrylate and challenged with 2% trimethylolpropane triacrylate; animals induced with the other test chemicals did not have cross reactions with trimethylolpropane triacrylate.

Parker *et al.* (1985) immunized outbred male and female Hartley guinea pigs (number not specified) with subcutaneous injection into the footpad and the nape of the neck with 0.1 mL of an emulsion containing trimethylolpropane triacrylate in ethanol:saline (1:4) in FCA. The total trimethylolpropane triacrylate dose was 11.5 µmol. Skin tests of 0.02 mL of 0.25% or 0.5% trimethylolpropane triacrylate in acetone:olive oil (4:1) were then applied to the shaved flank of the guinea pig, and reactions were recorded at 24, 48, 72, and 96 hours. Skin reactions were graded on a scale of 0 (no reaction) to 3 (severe reaction). On day 7, the reactions for both concentrations were mild at 24 hours and moderate at 48 hours; the 24- and 48-hour reactions on day 14 were moderate. Parker *et al.* (1985) also conducted cross sensitivity tests with trimethylolpropane triacrylate on groups of five guinea pigs immunized with methyl acrylate, methyl vinyl ketone, 4-vinyl pyridine, pentaerythritol triacrylate, and trimethylolpropane triacrylate in FCA. Cross sensitivity to trimethylolpropane triacrylate occurred in guinea pigs immunized with methyl acrylate, methyl vinyl ketone, and pentaerythritol triacrylate; the reactions were 20% to 60%, 60% to 80%, and greater than 80%, respectively, relative to the sensitizer response.

A radioimmunoassay detected no anti-trimethylolpropane acrylate antibodies in the serum of outbred female Hartley guinea pigs immunized with trimethylolpropane triacrylate (Bull *et al.*, 1987). However, high concentrations of these antibodies were observed in the serum of guinea pigs immunized with bovine γ-globulin. Prior to analysis, the immunized serum dilutions were incubated with egg albumin and 0.5 µg/mL to 5 mg/mL trimethylolpropane triacrylate, methyl acrylate, or 4-vinyl pyridine for 2 hours. The amount of bound radiolabel decreased with increasing dose, indicating that the anti-trimethylolpropane triacrylate/bovine γ-globulin antibodies specifically reacted with trimethylolpropane triacrylate and cross-reacted with methyl acrylate.

In studies conducted by Bull *et al.* (1985), lymph nodes of trimethylolpropane triacrylate-immunized guinea pigs were examined for T-lymphocyte proliferation 4 to 6 days after application of 50 µmol trimethylolpropane triacrylate to the ear. The numbers of large pyroninophilic cells in the lymph nodes were significantly increased 4 to 6 days after epicutaneous dosing. A positive correlation was noted between skin contact reactions and increases in lymph node weight and T-lymphocyte proliferation.

Humans

A number of studies have reported the development of dermatitis, characterized by itching of the exposed skin followed by erythema and scaling upon prolonged exposure, after workplace exposure to compounds containing trimethylolpropane triacrylate. In many cases of exposure to industrial mixtures of acrylates, individuals displayed positive reactions to two or more acrylates, making it difficult to establish the potency of trimethylolpropane triacrylate alone (Anonymous, 1985). Irritant or allergic contact dermatitis and irritant contact conjunctivitis occurred in 15 of 19 workers who cured multifunctional acrylic monomers in ultraviolet-curable inks (Nethercott, 1978). Three workers did not have direct contact with the acrylic monomers and were apparently exposed to airborne vapors. Two workers with allergic contact dermatitis had positive vesicular skin reactions to a 48-hour skin patch test with 0.1% trimethylolpropane triacrylate.

After trimethylolpropane triacrylate and pentaerythritol triacrylate were introduced as components of radiation drying ink in an ink formulating facility, five of 26 workers developed eczematous dermatitis (Emmett, 1977). Four of the five affected workers had positive reactions to patch tests using 0.2% trimethylolpropane triacrylate in a varnish formulation or in solution in petrolatum; the fifth worker developed irritant dermatitis to undiluted polyfunctional acrylates. Björkner *et al.* (1980) reported the development of allergic dermatitis in six people who worked with ultraviolet-curable inks containing trimethylolpropane triacrylate for 3 to 32 weeks. All six had positive reactions to skin patch tests with 0.1% or 0.5% trimethylolpropane triacrylate in acetone. Seven of 10 workers exposed to ultraviolet-curable printing inks at a plastic food container manufacturing plant developed contact dermatitis; one person had a positive reaction for sensitization to 0.1% trimethylolpropane triacrylate in petrolatum (Nethercott *et al.*, 1983).

Four workers in a plastic floor manufacturing facility developed hand and face dermatitis a year after the introduction of a varnish with an aziridine-based hardener containing 3% to 5% trimethylolpropane triacrylate (Dahlquist *et al.*, 1983). The workers had positive reactions to skin patch tests with trimethylolpropane triacrylate in acetone at 0.0001% (1/4), 0.03% (3/4), and 0.1% (4/4), with the most severe reactions occurring in the worker who reacted to the 0.0001% formulation. Contact dermatitis occurred in 13 of 51 workers at a wallpaper printing company following the introduction of a water-based ink containing a polyfunctional aziridine hardening agent, of which trimethylolpropane triacrylate was a component (Garabrant, 1985). A worker in optical fiber manufacturing developed dermatitis of the hands, face, and eyelids after 2 years of exposure to ultraviolet-curing acrylate resin coatings containing trimethylolpropane triacrylate and urethane acrylate; the dermatitis cleared within a week after the exposure ended (Maurice and Rycroft, 1986). This worker had positive reactions to 2- and 4-day skin patch tests with 0.01% (2-day test only) and 0.1% trimethylolpropane triacrylate in petrolatum.

Cofield *et al.* (1985) reported that five of 44 patients administered a skin patch test with 0.5% trimethylolpropane triacrylate in petrolatum demonstrated irritant reactions which diminished after 1 week; concentrations of 0.001% to 0.19% induced no reactions. A group of 50 patients had no reaction to a skin patch test of a cross-linking agent (containing trimethylolpropane triacrylate and aziridine) at a concentration of 0.1% in petrolatum.

Skin sensitivity and photo patch testing of 0.2% trimethylolpropane triacrylate in petrolatum was performed on 47 employees of a citrus juice bottling plant who were exposed to ultraviolet-cured printing inks (NIOSH, 1987). All 47 workers had positive reactions to one or both tests. Because few workers showed skin sensitization to trimethylolpropane triacrylate, the past skin reactions were considered to be irritant, not allergic, reactions to the inks and their components.

Reproductive and Developmental Toxicity

No data on the reproductive or teratogenic effects of trimethylolpropane triacrylate in animals or humans were found in the literature.

Carcinogenicity

Experimental Animals

No neoplasms occurred in 50 male C3H/HeJ mice administered 100 mg/kg dermal applications of a solution of 5% trimethylolpropane triacrylate in mineral oil to the interscapular region twice per week for up to 80 weeks (Andrews and Clary, 1986). Ten percent of the group was examined histologically. Slight epilation of the skin was noted at the site of application; in addition, acanthosis of the epidermis occurred in 46 mice and fibrosis of the dermis in 38 mice. No lesions were noted in control mice.

Humans

No epidemiology studies or case reports associating trimethylolpropane triacrylate exposure with cancer risk in humans were found in the literature.

Related Compounds

In a series of studies of eight multifunctional alkyl acrylates performed by Celanese Corporation, Inc., groups of 50 male C3H/HeJ mice were given dermal applications twice weekly for up to 80 weeks (Andrews and Clary, 1986). No increases in the incidences of skin or visceral tumors were induced by trimethylolpropane triacrylate; trimethylolpropane trimethacrylate; 1,6-hexanediol diacrylate; tripropyleneglycol diacrylate; or triethyleneglycol dimethacrylate. However, pentaerythritol triacrylate, triethyleneglycol diacrylate, and tetraethyleneglycol diacrylate showed some potential for carcinogenicity when administered at doses of 100 mg/kg in mineral oil. Pentaerythritol triacrylate induced lymphoma with spleen or lymph node involvement in six mice. However, these lesions were not verified in subsequent examinations. Triethyleneglycol diacrylate induced skin tumors in six mice and lymphomas in four mice; tetraethyleneglycol diacrylate induced skin tumors in six mice. However, in 78-week dermal carcinogenicity studies, neither triethyleneglycol diacrylate (0.05%, 0.1%, or 0.5% in acetone) nor triethyleneglycol dimethacrylate (5%, 25%, or 50% in acetone) were carcinogenic in male C3H/HeNHsd mice (Van Miller *et al.*, 2003).

In another study, groups of 40 male C3H/HeJ mice were given dermal applications of 5 mg (approximately 200 mg/kg) neopentylglycol diacrylate or 3 mg (approximately 120 mg/kg) pentaerythritol triacrylate in acetone

three times weekly for the lifetime of the animals (DePass *et al.*, 1995). Among mice administered neopentylglycol diacrylate, five had sking papilloma and three had skin carcinoma. No skin neoplasms were observed in pentaerythritol triacrylate-treated mice.

GENETIC TOXICITY

Trimethylolpropane triacrylate monomer (79% pure) demonstrated weak mutagenic activity in *Salmonella typhimurium* strain TA1535 in the presence of hamster liver S9 activation enzymes (Cameron *et al.*, 1991); negative results were obtained with other strains, with and without induced rat or hamster liver S9 enzymes. Tests of the cross-linked polymer (with molecular weights ranging up to 1,000,000) at concentrations up to 6,666 µg/plate showed no mutagenic activity in any of several strains of *S. typhimurium*, with or without exogenous metabolic activation derived from induced rat liver (Thompson *et al.*, 1991).

Trimethylolpropane triacrylate monomer (0.6, 0.65, and 0.7 µg/mL) was tested in L5178Y mouse lymphoma cells without exogenous metabolic activation for induction of forward mutations at the *tk* locus, chromosomal aberrations, and micronuclei (Dearfield *et al.*, 1989). Significant dose-related increases in frequencies were observed for all three endpoints; mutant *tk* colonies were almost exclusively small in size, indicative of the induction of large deletions or other chromosomal alterations rather than point mutations. Further supporting a clastogenic mechanism for the mutagenicity of trimethylolpropane triacrylate are the results of comparative studies reported by Moore *et al.* (1989) which were conducted with the *tk* locus in L5178Y cells and the hemizygous *hgprt* locus in Chinese hamster ovary cells as targets. Although significant increases in mutant *tk* small colonies were observed in the L5178Y cells, no increase in the frequency of *hgprt* mutant Chinese hamster ovary cells was observed. The authors suggested that the hemizygous nature of the *hgprt* locus renders it insensitive to the action of clastogens. Results reported by Cameron *et al.* (1991) confirmed the positive results with the trimethylolpropane triacrylate monomer in mutagenicity tests using L5178Y mouse lymphoma cells without S9 activation. The trimethylolpropane triacrylate cross-linked polymer was also tested in L5178Y mouse lymphoma cells for induction of mutations in the presence and absence of exogenous metabolic activation; the results were negative over a range of concentrations up to 1,392 µg/mL (Thompson *et al.*, 1991). Results of additional tests with the trimethylolpropane triacrylate cross-linked polymer that were part of the comprehensive investigation by Thompson *et al.* (1991) showed no induction of unscheduled DNA synthesis in rat primary hepatocyte cultures and no increase in the incidence of chromosomal aberrations in the bone marrow of male or female rats administered the polymer as a slurry by oral gavage in amounts up to 16 mL/kg. The trimethylolpropane triacrylate monomer was positive in tests for induction of chromosomal aberrations in Chinese hamster ovary cells and L5178Y mouse lymphoma cells, producing dose-related increases in the frequency of aberrations in both systems at doses up to 0.7 µg/mL (Moore *et al.*, 1989).

STUDY RATIONALE

Trimethylolpropane triacrylate was nominated by the National Cancer Institute for study due to its high production volume and use, the potential for human exposure, and the lack of adequate chronic toxicity and carcinogenicity data. It was also chosen as a representative of the multifunctional acrylate class. Trimethylolpropane triacrylate is a suspected carcinogen as a member of this class of compounds; some members of this class have been shown to be carcinogenic to mice in dermal studies.

The major route of human exposure to trimethylolpropane triacrylate is via the skin. At the time of study design, the Tg.AC mouse model was showing promise relative to carcinogenicity testing (hazard identification) via dermal exposure. Efforts were under way to to assess more fully the potential of the model. Therefore, it was decided to conduct initial studies in the Tg.AC mouse model and, upon completion of the studies, assess the findings in light of updated information about the model. Importantly, this process allowed assessment of studies in the Tg.AC mouse model in a completely prospective manner.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Trimethylolpropane Triacrylate

Trimethylolpropane triacrylate was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (01031AW), which was used throughout the studies. Identity, moisture content, purity, and stability analyses were conducted by the analytical chemistry laboratories and the study laboratory (Battelle Columbus Laboratories, Columbus, OH). Reports on analyses performed in support of the trimethylolpropane triacrylate studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a colorless to yellow viscous liquid, was identified as trimethylolpropane triacrylate by the analytical chemistry laboratory using infrared spectroscopy and by proton and carbon-13 nuclear magnetic resonance spectroscopy and by the study laboratory using infrared spectrometry. The analytical laboratories and the study laboratory determined moisture content using Karl Fischer titration and purity using elemental analyses, gas chromatography, high-performance liquid chromatography (HPLC), and HPLC with mass spectrometry (HPLC/MS). Karl Fischer titration indicated approximately 747 ppm water. Elemental analyses for carbon, hydrogen, and oxygen were in agreement with the theoretical values for trimethylolpropane triacrylate. Gas chromatography indicated one major peak and two impurities with areas of 6.5% and 3.4% relative to the major peak area. HPLC indicated a major peak and five impurities with a combined area of 22.2%. HPLC/MS indicated 10 impurities including the five impurities found by HPLC. These impurities included four structurally related acrylates or adducts: trimethylolpropane diacrylate, trimethylolpropane triacrylate acrylic acid adduct, trimethylolpropane triacrylate-trimethylolpropane monoacrylate adduct, and trimethylolpropane triacrylate-trimethylolpropane diacrylate adduct. The overall purity of lot 01031AW was estimated to be approximately 80%.

To ensure stability, the bulk chemical was stored at room temperature, protected from light, in amber glass bottles with Teflon®-lined lids. Stability was monitored throughout the studies with gas chromatography. No degradation of the bulk chemical was detected.

12-*O*-Tetradecanoylphorbol-13-acetate

12-*O*-Tetradecanoylphorbol-13-acetate was obtained from Sigma Chemical Company (St. Louis, MO) in one lot (48H1178) for use in the 6-month study. Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC). The bulk chemical was stored in its original containers, protected from light, at –20° C or less.

The chemical was identified as 12-*O*-tetradecanoylphorbol-13-acetate by infrared and proton NMR spectroscopy. The purity was determined with HPLC, which indicated a major peak, one impurity peak with an area of approximately 0.11% of the total peak area, and two minor impurities with areas less than 0.1% of the total peak area. The overall purity was determined to be greater than 99%.

Acetone

Acetone was obtained in two lots from Honeywell Burdick and Jackson (Muskegon, MI) (lots BK792 and BL631) and in five lots from Spectrum Chemical Manufacturing Corporation (Gardena, CA) (lots JE342, KP206, LS0051, MI0172, and NE0173). Lots BK792, BL631, and JE342 were used in the 2-week studies, lots KP206 and LS0051 were used in the 3-month studies, and lots MI0172 and NE0173 were used in the 6-month study. Identity and purity analyses of lots BL631 and JE342 and all lots used in the 3- and 6-month studies were conducted by the analytical chemistry laboratory (Midwest Research Institute, Kansas City, MO) and the study laboratory.

The chemical, a clear liquid, was identified as acetone by the analytical chemistry laboratory (lots BL631, JE342, KP206, and LS0051) or the study laboratory (lots MI0172 and NE0173) using infrared spectroscopy. The purity was analyzed by the analytical chemistry laboratory (lots BL631, JE342, KP206, and LS0051) or the

study laboratory (lots MI0172 and NE0173) using gas chromatography. No significant impurities were detected in any lot. The overall purity of each lot was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored in amber glass bottles at room temperature. Stability was monitored with gas chromatography. No degradation of the acetone was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared twice (2-week studies) or every 4 weeks by mixing trimethylolpropane triacrylate and acetone (Table H2). The dose formulations were stored for up to 35 days at room temperature in amber glass bottles with Teflon®-lined lids or in amber or clear glass vials with Teflon® septa at –20° C or less. Positive control formulations for the 6-month study were prepared twice by mixing 12-*O*-tetradecanoylphorbol-13-acetate with acetone to provide a concentration of 12.5 µg/mL.

Stability studies of the 6.25 and 100 mg/mL dose formulations for the 2-week studies as well as 50 and 400 µg/mL dose formulations were performed by the study laboratory with gas chromatography. Stability was confirmed for at least 35 days for dose formulations stored in amber glass bottles with Teflon®-lined lids or septa, with minimal headspace, at temperatures up to 25° C and for 3 hours under animal room conditions, periodically or continually exposed to air and light.

Periodic analyses of the dose formulations of trimethylolpropane triacrylate were conducted by the study laboratory using gas chromatography. The dose formulations were analyzed once during the 2-week studies; at the beginning, midpoint, and end of the 3-month studies; and approximately every 8 or 12 weeks during the 6-month study (Tables H3 through H5). All dose formulations analyzed and used for dosing were within 10% of the target concentrations. Animal room samples were also analyzed periodically. During the 2-week studies, four of five animal room samples for rats and all samples for mice were within 10% of the target concentrations. During the 3-month studies, 12 of 15 animal room samples for rats and 14 of 15 for mice were within 10% of the target concentrations. During the 6-month study, all five animal room samples were within 10% of the target concentrations. The positive control formulations were analyzed by the analytical chemistry laboratory using HPLC with a system similar to that described for the positive control purity analysis and were found to be within 10% of the target concentration.

2-Week Studies

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 11 days (rats) or 12 days (mice) and were 6 weeks old on the first day of the studies. Before the studies began, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Groups of five male and five female rats and mice received dermal applications of 0, 12.5, 25, 50, 100, or 200 mg trimethylolpropane triacrylate/kg body weight in acetone 5 days per week for 16 days; the dosing volumes were 0.5 mL/kg body weight for rats and 2 mL/kg for mice. Feed and water were available *ad libitum*. Rats and mice were housed individually. The animals were weighed initially, on day 8, and at the end of the studies; clinical findings were recorded daily. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations were performed on the skin (site of application) of all rats and mice and the thymus of all mice.

3-Month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to trimethylolpropane triacrylate and to determine the appropriate doses to be used in the 6-month study.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services. On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 11 to 14 days and were 6 weeks old on the first day of the studies. Before the studies began, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. During week 4 and at the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix J).

Groups of 10 male and 10 female rats and mice received dermal applications of 0, 0.75, 1.5, 3, 6, or 12 mg/kg in acetone 5 days per week for 14 weeks; the dosing volumes were 0.5 mL/kg for rats and 2 mL/kg for mice. Additional groups of 10 male and 10 female rats designated for clinical pathology testing received the same doses for 23 days. Feed and water were available *ad libitum*. The feed was irradiated to reduce potential microbial contamination. Rats and mice were housed individually. The animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly and at necropsy. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 23 and from all core study rats and mice at the end of the studies for hematology and clinical chemistry (rats only) analyses. The animals were anesthetized with a mixture of carbon dioxide and oxygen. Samples for hematology analysis were placed in micro-collection tubes (Sarstedt, Inc., Nümbrecht, Germany) coated with potassium EDTA and inverted on an aliquot mixer to prevent clotting; samples for clinical chemistry evaluations were placed in serum separator tubes devoid of anticoagulant and centrifuged for the collection of serum. Hematocrit; hemoglobin concentration; erythrocyte, platelet, and leukocyte counts; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined with a Cell-Dyn® hematology analyzer (Abbott Diagnostics, Santa Clara, CA). Differential leukocyte counts and erythrocyte and platelet morphologies were determined microscopically from blood smears stained with a modified Wright-Giemsa stain. A Miller Disc was used to determine reticulocyte counts from smears prepared with blood stained with new methylene blue. For clinical chemistry analyses, serum samples were analyzed using a Hitachi 911® chemistry analyzer (Boehringer Mannheim, Indianapolis, IN) using commercially available reagents. The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm count and motility and vaginal cytology evaluations on core study rats and mice in the vehicle control and the 3, 6, and 12 mg/kg groups. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all core study rats and mice in the vehicle control and 12 mg/kg groups. The skin at the site of application was examined microscopically for core study animals in all groups. Table 1 lists the tissues and organs routinely examined.

6-Month Study

Study Design

Groups of 15 male and 15 female mice received dermal applications of 0, 0.75, 1.5, 3, 6, or 12 mg/kg in acetone 5 days per week for 28 weeks; the dosing volume was 3.3 mL/kg. Additional groups of 15 male and 15 female mice maintained as positive controls received dermal applications of 1.25 µg 12-*O*-tetradecanoylphorbol-13-acetate per 100 mL acetone 3 days per week for 28 weeks; the dosing volume was held constant at 100 µL.

Source and Specification of Animals

The foundation colony of FVB/N-TgN(v-Ha-*ras*) (i.e. Tg.AC) mice was reestablished in 1998 after observation of some Tg.AC mice that were nonresponsive to the tumor promoter 12-*O*-tetradecanoyl-phorbol-13-acetate after treatment with a defined exposure regimen known to induce skin papillomas at the site of application. The homozygous FVB/N-TgN(v-Ha-*ras*) (i.e. Tg.AC) colony was established using homozygous breeders showing an unequivocal pattern of bands of restriction enzyme digests of DNA (Thompson *et al.*, 1998, 2001; Honchel *et al.*, 2001) demonstrating a specific phenotype for the induction of papillomas by 12-*O*-tetradecanoyl-phorbol-13-acetate. All foundation colony breeders homozygous for the transgene are genotyped and test-mated with wildtype FVB mice and qualified as a homozygous transgenic mouse with a responder phenotype. Homozygous male breeders obtained from the litters of qualified homozygous foundation colonies were further qualified by test mating with wild type FVB/N mice and used to produce hemizygous Tg.AC mice. All Tg.AC hemizygous male and female mice are the product of this continuing quality control of the foundation and production colony.

Male and female Tg.AC hemizygous transgenic mice were obtained from the NIEHS/NTP colony at Taconic Laboratory Animals and Services. On receipt, the mice were 4 weeks old. Animals were quarantined for 11 days and were 6 weeks old on the first day of the studies. Before the study began, five male and five female mice were randomly selected for parasite evaluation and gross observation for evidence of disease. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix J).

Animal Maintenance

Mice were housed individually. The core study mice were housed in the same room with positive control mice and mice in the pentaerythritol triacrylate study (NTP, 2005). Feed and water were available *ad libitum*. The feed was irradiated to reduce potential microbial contamination. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix I.

Clinical Examinations and Pathology

The animals were observed twice daily and were weighed initially, weekly, and at the end of the study. Clinical findings were recorded weekly and at the end of the study.

In-life observations of papilloma formation on the skin were recorded weekly using the Toxicology Data Management System (TDMS). A papilloma was initially recorded as a mass. The observation "papilloma" was not entered into TDMS for a given animal until the first-observed mass was documented for 3 consecutive weeks. At the third observation, the mass (wart-like in appearance) was entered as a papilloma. Any new mass(es) appearing after the 3-week confirmation period for a given animal at a different site was entered into TDMS first as a mass until the third week, when it was entered as a papilloma. In a few instances, a papilloma that had been previously observed was missing, and therefore not recorded. Reappearance of a mass at a later time was entered into TDMS as a mass until the third observation week, when it was called a papilloma.

Necropsies and histopathologic examinations were performed on all core study mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and selected tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin for microscopic examination. The tissues selected for microscopic evaluation represented gross lesions as well as major organs and tumor target tissues for mice in chronic rodent bioassays. These tissues were examined in all core study mice. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Because the Tg.AC model was initially intended to be a skin reporter phenotype, less emphasis was placed on microscopic examination of internal organs. However, with time and for a variety of reasons, more interest developed relative to effects in internal organs. Thus a reduced tissue list (compared to the standard 2-year bioassay) was adopted that included all tissues that are common targets in NTP carcinogenicity studies. While the gross examination would likely detect any significant carcinogenic effects, it is possible that chemically induced nonneoplastic lesions occurred in organs not examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block

match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A quality assessment pathologist evaluated all slides from nine male and nine female mice per group randomly selected from the vehicle control and 12 mg/kg groups and from all mice that died early. Slides of all tumors and of all skin sites of application, which was the primary target tissue, were reviewed. Selected slides of other potential target organs, including the liver, spleen, and lymph nodes, were also reviewed.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Review chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were examined by the chairperson, an NTP pathologist, and a pathology working group. When the NTP Pathology Review consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist, and NTP Pathology Review chairperson. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Study Laboratory Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species F344/N rats $B6C3F_1$ mice	F344/N rats $B6C3F_1$ mice	Tg.AC [FVB/N-TgN(V-Ha-*ras*)] hemizygous mice
Animal Source Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies Rats: 11 days Mice: 12 days	Rats: 11 days (males) or 12 days (females) Mice: 13 days (females) or 14 days (males)	11 days
Average Age When Studies Began 6 weeks	6 weeks	6 weeks
Date of First Dose Rats: May 6, 1996 Mice: May 7, 1996	Rats: September 9 (male) or 10 (female), 1996 Mice: September 11 (female) or 12 (male), 1996	July 20, 1998
Duration of Dosing 5 days per week for 16 days	5 days per week for 14 weeks	Core study: 5 days per week for 28 weeks Positive control: 3 days per week for 28 weeks
Date of Last Dose Rats: May 21, 1996 Mice: May 22, 1996	Rats: December 10 (male) or 11 (female), 1996 Mice: December 12 (female) or 13 (male), 1996	Core study: January 27 (male) or 28 (female), 1999 Positive control: January 28, 1999
Necropsy Dates Rats: May 22, 1996 Mice: May 23, 1996	Rats: December 10 (male) or 11 (female), 1996 Mice: December 12 (female) or 13 (male), 1996	Core study: January 27 (male) or 28 (female), 1999 Positive control: January 28, 1999
Average Age at Necropsy 8 weeks	Rats: 19 weeks Mice: 19 (female) or 20 (male) weeks	33 weeks
Size of Study Groups 5 males and 5 females	10 males and 10 females	15 males and 15 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage 1	1	1

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Method of Animal Identification Tail tattoo	Tail tattoo	Tail tattoo
Diet NTP-2000 pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 2-week studies, except feed was irradiated	Same as 3-month studies
Water Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 2-week studies	Same as 2-week studies
Cages Polycarbonate (Lab Products, Inc., Maywood, NJ), changed at least once per week, rotated every 2 weeks	Same as 2-week studies	Same as 2-week studies
Bedding Sani-Chip® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed at least once per week	Same as 2-week studies, except bedding was irradiated	Same as 3-month studies
Cage Filters Spun-bonded DuPont 2024 polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 2-week studies	Same as 2-week studies
Racks Stainless steel, cleaned and rotated every 2 weeks	Same as 2-week studies	Same as 2-week studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses 0, 12.5, 25, 50, 100, or 200 mg/kg in acetone by dermal application (dosing volume 0.5 mL/kg for rats and 2 mL/kg for mice)	0, 0.75, 1.5, 3, 6, or 12 mg/kg in acetone by dermal application (dosing volume 0.5 mL/kg for rats and 2 mL/kg for mice)	Core study: 0, 0.75, 1.5, 3, 6, or 12 mg/kg in acetone by dermal application (dosing volume 3.3 mL/kg) Positive control: 1.25 µg 12-*O*-tetradecanoylphorbol-13-acetate/100 mL acetone by dermal application (dosing volume 100 µL)
Type and Frequency of Observation Animals were observed twice daily and were weighed initially, on day 8, and at the end of the studies. Clinical findings were recorded daily.	Animals were observed twice daily and were weighed initially, weekly, and at the end of the studies. Clinical findings were recorded weekly for core study animals.	Animals were observed twice daily and were weighed initially, weekly, and at the end of the study. Clinical findings were recorded weekly and at the end of the study. Observations of papilloma formation on the skin were recorded weekly.

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Method of Sacrifice		
Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy		
Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on core study mice. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.
Clinical Pathology		
None	Blood was collected from the retroorbital sinus of special study rats on days 4 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats only). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None
Histopathology		
Histopathologic examinations were performed on the skin (site of application) of all animals and the thymus of all mice.	Complete histopathologic examinations were performed on vehicle control and 12 mg/kg rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart with aorta, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin (site of application), spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The skin at the site of application was also examined in the remaining core study groups.	Histopathologic examinations were performed on all core study mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, heart, kidney, liver, lung, lymph nodes (mandibular, mediastinal, and mesenteric), ovary, pituitary gland, skin (site of application and inguinal), spleen, stomach (forestomach), testis with epididymis, thymus, thyroid gland, and uterus.

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Sperm Motility and Vaginal Cytology		
None	At the end of the studies, sperm samples were collected from male animals in the vehicle control and 3, 6, and 12 mg/kg groups for sperm count and motility evaluations. The following parameters were evaluated: spermatid heads per testis or cauda and per gram testis or cauda, and epididymal spermatozoal motility. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from females in the vehicle control and 3, 6, and 12 mg/kg groups for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, and B4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3 and B3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, and mammary gland) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3 and B3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, to animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion

at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F$_1$ mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidence or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

The weekly in-life skin papilloma counts were evaluated by the method of Dunson *et al.* (2000). The model separates effects on papilloma latency and multiplicity and accommodates important features of the data, including animal-to-animal variability in the expression of the transgene as reflected in the initial tumor counts. The two key parameters are γ_1, which measures the dose effect on incidence (number of animals with one or more papillomas during the study), and γ_2, which measures the dose effect on multiplicity (rate of appearance of additional papillomas after the initial papilloma has occurred). The model assumes that the rate (number of additional papillomas per time period) is exponentially increasing with respect to dose and that the rate remains constant across time.

More specifically, under the model, the increase in papilloma burden from one week to the next is assumed to be distributed as a Poisson random variable. The Poisson mean is assumed to depend on an animal-specific susceptibility variable, on exposure length, and on the dose. The rate of initial papilloma occurrence is assumed to be log-linear in time. The coefficients for time are levels of dose multiplied by γ_1 and the animal-specific susceptibility parameters. This implies that as the dose/time increases, the rate of occurrence for the first papilloma will increase exponentially relative to increases in dose/time. A value of zero for γ_1 implies that dose is not associated with incidence (or, equivalently, the length of the latency period prior to initial onset), leaving only animal-specific characteristics to explain any variability.

After the latency period (after the first papilloma occurs), the Poisson mean changes to a rate that is only dependent on dose (that is, no animal-specific rates or dependency with time). More explicitly, the rate of occurrence of additional papillomas is assumed to be log-linear in time. A value of zero for γ_2 implies that dose is not associated with rate of additional papilloma occurrence. A non-zero value implies that the rate of additional papillomas increases with dose in a proportional fashion.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous

stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses.

Quality Assurance Methods

The 3- and 6-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 6-month studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Report.

Genetic Toxicology

The genetic toxicity of trimethylolpropane triacrylate was assessed by testing the ability of the chemical to induce increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. The protocols for these studies and the results are given in Appendix C.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

Clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

RATS

2-WEEK STUDY

All rats survived to the end of the study (Table 2). Final mean body weights and body weight gains of all dosed groups were similar to those of the vehicle control groups. Dosed rats had irritation at the site of application; this clinical finding was most commonly seen in rats administered 50 mg/kg or greater.

TABLE 2
Survival and Body Weights of Rats in the 2-Week Dermal Study of Trimethylolpropane Triacrylate

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	97 ± 2	170 ± 6	73 ± 4	
12.5	5/5	97 ± 3	162 ± 6	66 ± 5	96
25	5/5	97 ± 2	167 ± 4	70 ± 4	98
50	5/5	96 ± 3	163 ± 8	67 ± 5	96
100	5/5	96 ± 2	163 ± 3	67 ± 3	96
200	5/5	96 ± 2	155 ± 4	59 ± 4	91
Female					
0	5/5	86 ± 2	124 ± 3	38 ± 1	
12.5	5/5	85 ± 1	124 ± 3	39 ± 1	100
25	5/5	86 ± 1	122 ± 1	35 ± 2	98
50	5/5	86 ± 2	122 ± 2	36 ± 2	98
100	5/5	87 ± 1	128 ± 5	41 ± 4	104
200	5/5	86 ± 1	118 ± 2	32 ± 2	95

[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

There were no biologically significant organ weight changes (Table F1). At necropsy, all dosed groups except 12.5 mg/kg females had crusts at the site of application (data not shown), and the incidence generally increased with increasing dose. Microscopically, lesions occurred at the site of application in all dosed groups (Table 3). Epidermal hyperplasia, hyperkeratosis, and sebaceous gland hyperplasia were found in most treated rats, and were characterized by increased layers of epidermal cells, thickened keratin layer, and prominence of sebaceous glands. Chronic active inflammation (mixed inflammatory cell infiltration) of the dermis also occurred in most rats. More severe lesions occurring generally at doses of 25 mg/kg or greater were ulcer (focal full-thickness necrosis of the epidermis), degeneration (vacuolar change of epidermal cells), parakeratosis (retention of nuclei in keratin layer), and accumulation of neutrophils (suppurative inflammation) in the degenerative and parakeratotic areas of the superficial epidermis. These lesions were not seen in the vehicle controls, and their severity generally increased with increasing dose.

Dose Selection Rationale: Lesions at the site of application occurred in all dose groups. Their severity generally increased with increasing dose and was moderate to marked in the higher dose groups. The incidences and severities of some of the changes (ulcers and suppurative inflammation) precluded use of 25 mg/kg or greater in a 3-month study; therefore, 0.75, 1.5, 3, 6, and 12 mg/kg were selected for use in the 3-month study in rats.

TABLE 3
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 2-Week Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Male						
Number Examined						
Microscopically	5	5	5	5	5	5
Epidermis, Hyperplasia[a]	0	5** (1.4)[b]	5** (1.8)	5** (2.6)	5** (3.2)	5** (3.4)
Hyperkeratosis	0	5** (1.6)	5** (1.2)	5** (2.4)	5** (2.0)	5** (2.0)
Sebaceous Glands, Hyperplasia	0	5** (2.0)	5** (1.8)	5** (2.8)	5** (2.2)	5** (2.0)
Dermis, Inflammation, Chronic Active	0	5** (1.6)	5** (2.0)	5** (2.8)	5** (2.8)	5** (3.4)
Ulcer	0	1 (2.0)	1 (1.0)	5** (2.2)	5** (2.6)	5** (2.8)
Epidermis, Degeneration	0	4* (1.3)	4* (1.0)	5** (2.4)	5** (2.6)	5** (3.2)
Parakeratosis	0	1 (1.0)	4* (1.8)	5** (2.6)	5** (3.2)	5** (3.8)
Epidermis, Inflammation, Suppurative	0	2 (1.5)	5** (1.8)	5** (3.0)	5** (3.0)	5** (3.8)
Female						
Number Examined						
Microscopically	5	5	5	5	5	5
Epidermis, Hyperplasia	0	5** (1.2)	5** (2.0)	5** (2.6)	5** (3.2)	5** (3.6)
Hyperkeratosis	0	5** (1.0)	5** (1.4)	5** (1.2)	5** (2.0)	3* (2.0)
Sebaceous Glands, Hyperplasia	0	5** (1.4)	5** (2.0)	5** (2.2)	5** (2.2)	5** (1.8)
Dermis, Inflammation, Chronic Active	0	3* (1.0)	5** (2.0)	5** (2.8)	5** (3.0)	5** (3.4)
Ulcer	0	0	4* (1.5)	5** (3.2)	5** (2.6)	5** (3.0)
Epidermis, Degeneration	0	1 (1.0)	5** (2.2)	5** (3.0)	5** (3.0)	5** (3.0)
Parakeratosis	0	3* (1.7)	5** (2.2)	5** (3.0)	5** (3.0)	5** (3.2)
Epidermis, Inflammation, Suppurative	0	1 (1.0)	4* (2.0)	5** (3.0)	5** (3.4)	5** (3.2)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

3-MONTH STUDY

All rats survived to the end of the study. Final mean body weights and body weight gains in dosed groups were similar to those of the vehicle controls (Table 4 and Figure 1). Irritation at the site of application was noted in five males and all females administered 12 mg/kg.

Hematology and clinical chemistry data for rats in the 3-month dermal study of trimethylolpropane triacrylate are listed in Table E1. Segmented neutrophil counts were increased in 12 mg/kg males and females at week 14; the increase in males was significant. These increases would be consistent with the suppurative dermatitis observed microscopically. Decreased lymphocyte counts in males at week 14 would be consistent with a stress-related response.

Absolute and relative thymus weights of 12 mg/kg males, absolute thymus weights of 0.75, 6, and 12 mg/kg females, and relative thymus weights of 0.75 and 12 mg/kg females were significantly less than those of the vehicle controls (Table F2). Other organ weight changes were not considered biologically significant. Left testis weights were significantly decreased in 12 mg/kg male rats (Table G1). There were no significant differences in vaginal cytology parameters between dosed and vehicle control females (Table G2).

TABLE 4
Survival and Body Weights of Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	88 ± 3	272 ± 6	183 ± 7	
0.75	10/10	89 ± 3	288 ± 6	199 ± 5	106
1.5	10/10	87 ± 3	270 ± 8	183 ± 9	99
3	10/10	88 ± 3	263 ± 5	174 ± 4	97
6	10/10	87 ± 3	267 ± 6	180 ± 6	98
12	10/10	89 ± 4	259 ± 7	170 ± 7	95
Female					
0	10/10	82 ± 2	171 ± 3	89 ± 3	
0.75	10/10	81 ± 2	164 ± 2	83 ± 3	96
1.5	10/10	83 ± 2	170 ± 2	87 ± 2	99
3	10/10	82 ± 2	173 ± 3	91 ± 3	101
6	10/10	82 ± 2	164 ± 2	81 ± 2	96
12	10/10	83 ± 2	169 ± 3	86 ± 4	99

[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

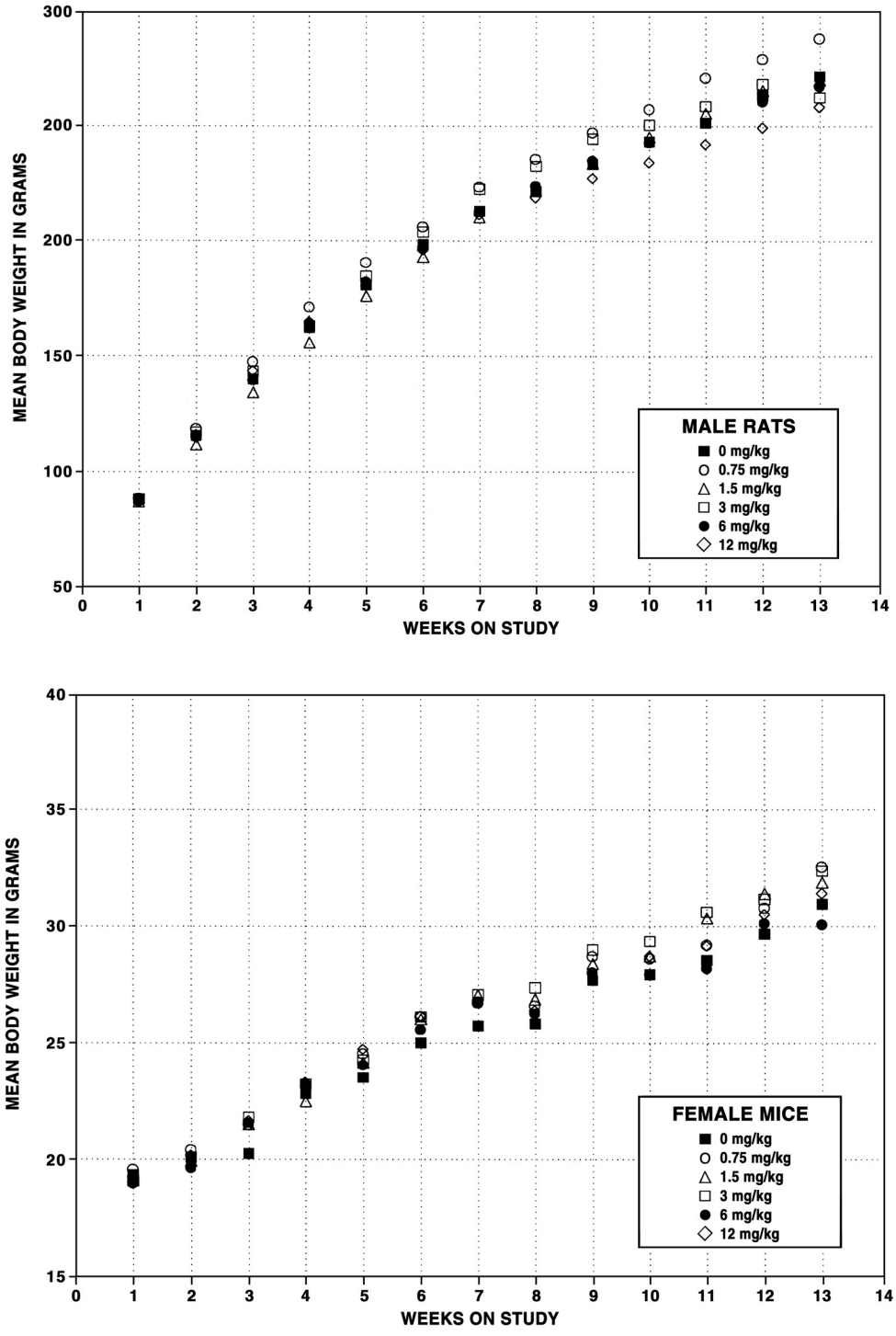


FIGURE 1
Growth Curves for Male and Female F344/N Rats
Administered Trimethylolpropane Triacrylate Dermally for 3 Months

Microscopically, the primary changes at the site of application were epidermal hyperplasia, degeneration, and necrosis, and chronic active inflammation of the dermis (Tables 5, D1, and D2). Epidermal hyperplasia occurred in all dosed groups of males and in 3 mg/kg or greater females. The severity of epidermal hyperplasia was minimal to mild and characterized by focally extensive to diffuse increased thickness of the epidermis, from the normal of one to three cell layers thick to four to six layers. Hyperplasia was accompanied by minimal to mild increased thickness of the superficial keratin layer (hyperkeratosis). Degeneration was seen in 1.5 mg/kg or greater males and 3 mg/kg or greater females. This lesion was a minimal to mild focal change consisting of intraepidermal vacuolization, presumably due to intra- or intercellular fluid accumulation. Epidermal necrosis was present in some 12 mg/kg females, although a dose response was not clear. Necrosis consisted of partial to full thickness coagulative change of the epidermis and was likely a sequela of degeneration. Intraepidermal infiltration of neutrophils (suppurative inflammation) often accompanied degeneration or necrosis of the epidermis. A mixed inflammatory cell infiltrate (chronic active inflammation) was present in the dermis of all dosed groups; the incidence increased with dose and the severity was increased in 12 mg/kg rats. Sebaceous glands at the site of application were slightly enlarged and prominent (hyperplasia) in animals administered 1.5 mg/kg or greater.

TABLE 5
Incidences of Selected Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
Numbered Examined						
Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia[a]	0	4* (1.0)[b]	7** (1.0)	10** (1.2)	10** (1.1)	10** (1.6)
Epidermis, Degeneration	0	0	4* (1.0)	7** (1.0)	9** (1.0)	8** (1.9)
Dermis, Inflammation, Chronic Active	0	1 (1.0)	3 (1.0)	6** (1.0)	10** (1.0)	10** (2.1)
Hyperkeratosis	0	0	5* (1.0)	10** (1.2)	10** (1.3)	10** (1.6)
Epidermis, Inflammation, Suppurative	0	0	0	0	0	4* (1.5)
Sebaceous Gland, Hyperplasia	0	0	5* (1.0)	10** (1.2)	10** (1.5)	10** (2.6)
Female						
Numbered Examined						
Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia	0	0	0	7** (1.0)	10** (1.1)	10** (1.4)
Epidermis, Degeneration	0	0	0	4* (1.0)	7** (1.0)	10** (2.0)
Epidermis, Necrosis	0	0	0	0	0	5* (2.0)
Dermis, Inflammation, Chronic Active	1 (1.0)	2 (1.0)	1 (1.0)	9** (1.0)	8** (1.0)	10** (1.8)
Hyperkeratosis	0	0	3 (1.0)	9** (1.0)	10** (1.1)	10** (2.0)
Epidermis, Inflammation, Suppurative	0	0	0	0	0	6** (1.3)
Sebaceous Gland, Hyperplasia	0	1 (1.0)	6** (1.0)	9** (1.1)	10** (1.3)	10** (2.1)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

MICE

2-WEEK STUDY IN B6C3F$_1$ MICE

All mice survived to the end of the study (Table 6). The final mean body weight gain of 200 mg/kg males was significantly less than that of the vehicle controls; final mean body weights of 100 and 200 mg/kg females were significantly increased. Irritation at the site of application occurred in all dosed males, all 100 and 200 mg/kg males, and one 50 mg/kg female.

TABLE 6
Survival and Body Weights of B6C3F$_1$ Mice in the 2-Week Dermal Study of Trimethylolpropane Triacrylate

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	21.8 ± 0.3	25.2 ± 0.5	3.4 ± 0.3	
12.5	5/5	21.9 ± 0.4	25.6 ± 0.3	3.7 ± 0.6	101
25	5/5	21.4 ± 0.3	25.0 ± 0.4	3.6 ± 0.2	99
50	5/5	22.5 ± 0.6	25.5 ± 0.6	3.1 ± 0.2	101
100	5/5	22.3 ± 0.6	23.7 ± 1.5	1.4 ± 1.4	94
200	5/5	22.4 ± 0.5	23.1 ± 1.3	0.6 ± 1.5*	91
Female					
0	5/5	18.7 ± 0.4	21.0 ± 0.5	2.3 ± 0.4	
12.5	5/5	18.4 ± 0.5	21.3 ± 0.5	2.9 ± 0.3	101
25	5/5	18.3 ± 0.3	22.0 ± 0.3	3.7 ± 0.6	105
50	5/5	18.1 ± 0.2	21.5 ± 0.2	3.4 ± 0.3	102
100	5/5	19.5 ± 0.4	22.5 ± 0.3*	2.9 ± 0.5	107
200	5/5	19.3 ± 0.3	22.4 ± 0.4*	3.1 ± 0.2	107

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

Thymus weights of male mice administered 50 mg/kg or greater were significantly decreased (Table F3). At necropsy, crusts were observed grossly at the site of application in 50 mg/kg and greater males and in 100 and 200 mg/kg females (data not shown). Microscopically, lesions occurred at the site of application in all dosed groups (Table 7). Hyperplasia of the epidermis, hyperkeratosis, dermal chronic active inflammation, and sebaceous gland hyperplasia occurred in most dosed mice; these lesions were characterized by increased layers of epidermal cells, a thickened keratin layer, dermal infiltration of mixed inflammatory cells, and prominence of sebaceous glands. More severe lesions occurring generally at higher doses were ulcer (focal full-thickness necrosis of the epidermis), degeneration (vacuolar change of epidermal cells), parakeratosis (retention of nuclei in keratin layer), and accumulation of neutrophils (suppurative inflammation) in the degenerative and parakeratotic areas of the superficial epidermis. These lesions were not seen in the vehicle controls, and their severity generally increased with increasing dose. Male mice administered 100 or 200 mg/kg had increased incidences of thymic atrophy, characterized by depletion of cortical lymphocytes.

Dose Selection Rationale: Lesions at the site of application occurred in all dose groups. Their severity generally increased with increasing dose and was moderate to marked in the higher dose groups. The presence of ulceration and suppurative inflammation precluded use of 50 mg/kg or greater for males and 100 mg/kg or greater for females. Lesions were not markedly different between 12.5 and 25 mg/kg males and 12.5, 25, and 50 mg/kg females. However, the combined incidences and severity of the lesions in each of these dose groups represented the upper end of a dose acceptable for use in a 3-month study. Therefore, 12 mg/kg was selected as the highest dose for the 3-month study.

TABLE 7
Incidences of Selected Nonneoplastic Lesions in B6C3F$_1$ Mice in the 2-Week Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Male						
Skin, Site of Application[a]	5	5	5	5	5	5
Epidermis, Hyperplasia[b]	0	5** (1.6)[c]	5** (2.2)	5** (2.2)	5** (2.8)	5** (3.6)
Hyperkeratosis	0	5** (1.4)	4* (1.3)	5** (1.8)	3* (1.7)	3* (1.7)
Dermis, Inflammation, Chronic Active	0	5** (1.8)	5** (1.8)	5** (2.6)	5** (3.0)	5** (2.8)
Sebaceous Gland, Hyperplasia	0	5** (2.2)	5** (1.6)	2 (2.5)	5** (1.6)	1 (1.0)
Ulcer	0	0	0	2 (1.0)	2 (1.0)	5** (2.8)
Epidermis, Degeneration	0	3* (1.0)	4* (1.0)	1 (1.0)	1 (1.0)	1 (1.0)
Parakeratosis	0	0	0	2 (2.0)	4* (2.3)	5** (2.4)
Epidermis, Inflammation, Suppurative	0	5** (1.4)	2 (1.0)	4* (1.8)	4* (1.8)	5** (3.0)
Thymus	5	5	5	5	5	5
Atrophy	0	0	0	0	3* (2.0)	3* (2.0)
Female						
Skin, Site of Application	5	5	5	5	5	5
Epidermis, Hyperplasia	0	5** (1.6)	5** (1.8)	5** (2.2)	5** (2.8)	5** (3.0)
Hyperkeratosis	0	3* (1.0)	4* (1.5)	5** (1.8)	4** (1.8)	4* (1.8)
Dermis, Inflammation, Chronic Active	0	5** (2.2)	5** (2.2)	5** (2.0)	5** (2.4)	5** (2.8)
Sebaceous Gland, Hyperplasia	0	5** (2.8)	5** (2.4)	5** (2.2)	5** (2.2)	5** (2.2)
Ulcer	0	0	1 (1.0)	0	3* (2.3)	3* (1.3)
Epidermis, Degeneration	0	2 (1.0)	3* (1.3)	1 (1.0)	3* (1.3)	4* (1.5)
Parakeratosis	0	1 (2.0)	2 (1.0)	1 (2.0)	3* (1.7)	5** (2.0)
Epidermis, Inflammation, Suppurative	0	1 (1.0)	4* (1.5)	2 (1.0)	5** (2.0)	5** (2.0)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

3-Month Study in B6C3F$_1$ Mice

All animals survived to the end of the study. Final mean body weights and body weight gains of dosed groups were similar to those of the vehicle controls (Table 8 and Figure 2). Irritation at the site of application occurred in male and female mice administered 12 mg/kg.

Hematology data for mice in the 3-month study of trimethylolpropane triacrylate are listed in Table E2. Increased segmented neutrophil counts in the 12 mg/kg males and females at study termination were consistent with the suppurative dermatitis observed microscopically.

Organ weights of dosed groups of mice were generally similar to those of the vehicle controls (Table F4). Trimethylolpropane triacrylate administration did not affect reproductive endpoints in males (Table G3). Although the relative length of time spent in the estrous stages differed significantly from the vehicle controls in the 6 and 12 mg/kg groups, the differences were not considered biologically significant (Table G4).

Table 8
Survival and Body Weights of B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	22.6 ± 0.4	36.5 ± 0.7	13.9 ± 0.6	
0.75	10/10	22.5 ± 0.4	34.5 ± 0.7	12.0 ± 0.5	95
1.5	10/10	22.2 ± 0.4	35.6 ± 1.1	13.4 ± 0.8	98
3	10/10	22.4 ± 0.5	35.8 ± 0.8	13.4 ± 0.9	98
6	10/10	22.3 ± 0.4	34.2 ± 0.7	11.9 ± 0.4	94
12	10/10	22.6 ± 0.5	34.6 ± 0.7	12.0 ± 0.9	95
Female					
0	10/10	19.4 ± 0.4	31.0 ± 1.2	11.6 ± 0.9	
0.75	10/10	19.6 ± 0.4	32.6 ± 0.7	13.0 ± 0.6	105
1.5	10/10	19.3 ± 0.4	31.9 ± 1.3	12.6 ± 1.2	103
3	10/10	19.1 ± 0.4	32.4 ± 1.2	13.3 ± 0.9	105
6	10/10	19.0 ± 0.4	30.1 ± 0.7	11.1 ± 0.6	97
12	10/10	19.3 ± 0.2	31.4 ± 1.1	12.2 ± 1.0	101

[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

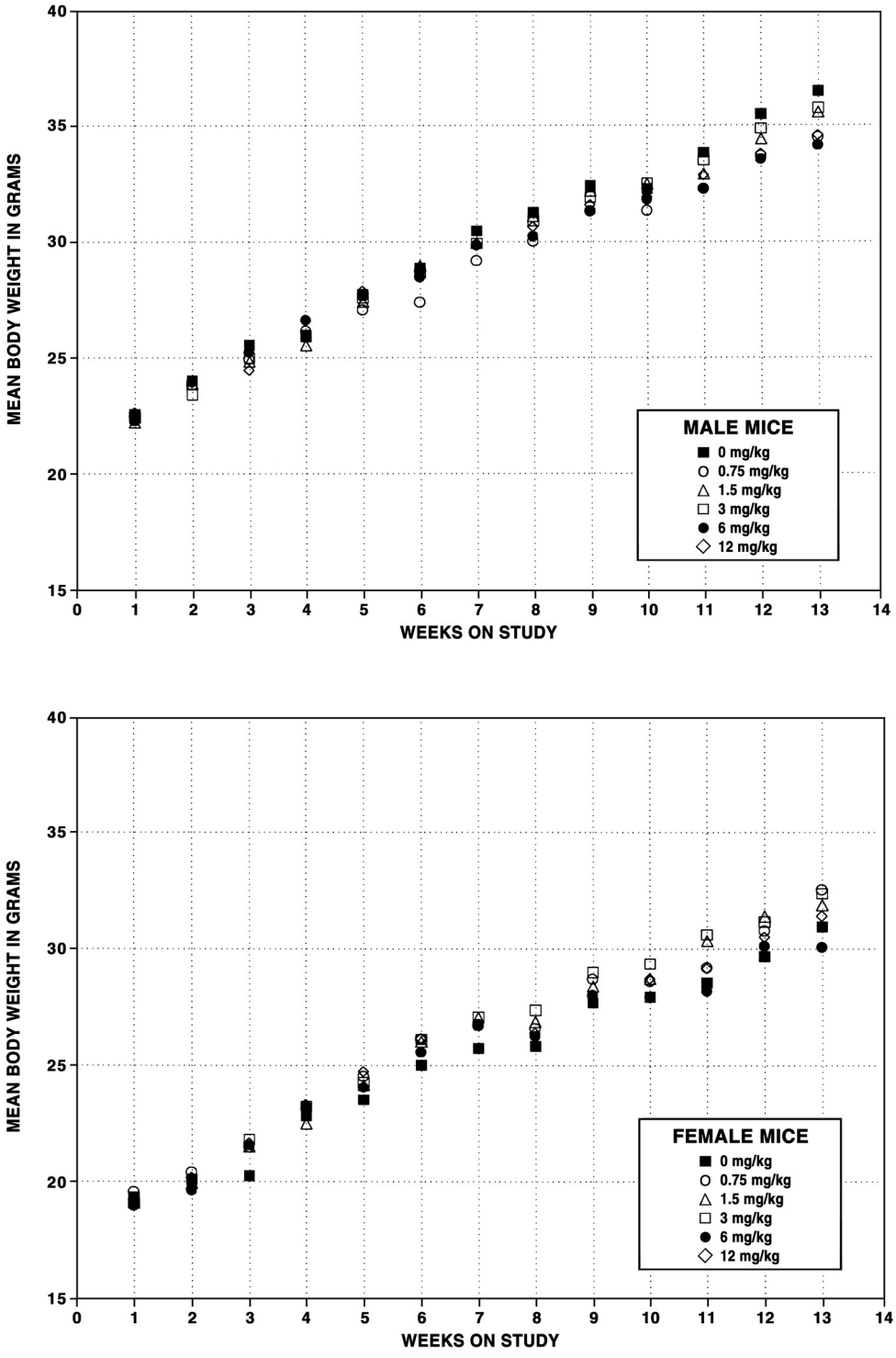


FIGURE 2
Growth Curves for Male and Female B6C3F$_1$ Mice
Administered Trimethylolpropane Triacrylate Dermally for 3 Months

Similar to the changes in rats, the primary microscopic changes at the site of application in mice were epidermal hyperplasia, degeneration, and necrosis, and chronic active inflammation of the dermis (Tables 9, D3, and D4). The incidences and severities of epidermal hyperplasia generally increased with increasing dose in the 1.5 mg/kg or greater groups (Plates 1 to 7). Severity was minimal to mild and was characterized by focally extensive to diffuse increased thickness of the epidermis from the normal one to three cell layers to four to six cell layers (Plates 3 to 7). Hyperplasia was accompanied by minimal to mild increases in the thickness of the superficial keratin layer (hyperkeratosis). Minimal to mild epidermal degeneration was diagnosed in several mice administered 3 mg/kg (males) or 6 mg/kg (females) or greater (Plates 5 to 7). This degeneration was a focal change consisting of intraepidermal vacuolization, presumably due to intra- or intercellular fluid accumulation. Minimal to mild epidermal necrosis was diagnosed with increasing incidence and severity in 1.5 mg/kg or greater male mice (Plates 6 and 7); in females, a similar trend occurred in the 3 mg/kg or greater groups. Necrosis consisted of partial to full thickness coagulative change of the epidermis and was likely a pathogenic sequela of degeneration. A mixed inflammatory cell infiltrate (chronic active inflammation) was present in most animals in the 1.5 mg/kg or greater groups, with dose-dependent increases in incidences and severities (Plates 3 to 6). Neutrophils were more prominent in areas of degeneration or necrosis (suppurative inflammation), and slight superficial dermal fibrosis was seen in some 12 mg/kg males and females and one 6 mg/kg female. Sebaceous glands at the site of application were slightly enlarged and prominent (hyperplasia; Plates 5 to 7) in 3 mg/kg or greater males and females.

Dose Selection Rationale: There were no effects on survival or body weights of $B6C3F_1$ mice treated with trimethylolpropane triacrylate in the 3-month study. In selecting doses for a 2-year dermal bioassay based on findings in 3-month studies, dose levels containing any severity of ulceration and necrosis or other changes (e.g. inflammation or hyperplasia) in the skin (site of application) that are moderate to marked are generally avoided. Therefore, because of the presence of mild necrosis and minimal to mild degeneration and suppurative inflammation, 12 mg/kg would not have been selected for use in a 2-year bioassay. These pathological alterations are consistent with the irritation identified grossly in the 12 mg/kg group. The necrosis and degeneration occurred in fewer animals and/or at lesser severity in the 6 mg/kg groups and were rare in the lower dose groups. Because doses were being selected for Tg.AC mice and for a 6-month study, five dose levels were used to allow for a margin of error, ranging from doses that produced no lesions to 12 mg/kg.

TABLE 9
Incidences of Selected Nonneoplastic Lesions of the Skin (Site of Application) in B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
Number Examined						
Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia[a]	0	0	3 (1.0)[b]	10** (1.0)	10** (1.4)	10** (2.2)
Epidermis, Degeneration	0	0	0	4* (1.0)	8** (1.0)	9** (1.8)
Epidermis, Necrosis	0	0	1 (1.0)	1 (1.0)	2 (1.5)	7** (2.1)
Dermis, Inflammation, Chronic Active	1 (1.0)	0	2 (1.0)	10** (1.1)	9** (1.4)	10** (1.8)
Hyperkeratosis	0	0	3 (1.0)	8** (1.0)	8** (1.0)	10** (1.4)
Epidermis, Inflammation, Suppurative	0	0	1 (1.0)	0	1 (1.0)	8** (1.6)
Dermis, Fibrosis	0	0	0	0	0	7** (1.1)
Sebaceous Gland, Hyperplasia	0	0	0	9** (1.0)	10** (1.7)	10** (2.3)
Female						
Number Examined						
Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia	0	0	3 (1.0)	10** (1.0)	9** (1.1)	10** (1.3)
Epidermis, Degeneration	0	0	0	0	5* (1.0)	9** (1.6)
Epidermis, Necrosis	0	0	0	1 (1.0)	2 (2.0)	8** (2.0)
Dermis, Inflammation, Chronic Active	0	0	7** (1.0)	10** (1.1)	10** (2.0)	10** (1.8)
Hyperkeratosis	0	0	3 (1.0)	10** (1.0)	9** (1.0)	8** (1.1)
Epidermis, Inflammation, Suppurative	0	0	1 (1.0)	1 (1.0)	2 (1.5)	5* (1.4)
Dermis, Fibrosis	0	0	0	0	1 (1.0)	7** (1.6)
Sebaceous Gland, Hyperplasia	0	0	1 (1.0)	10** (1.0)	10** (2.0)	10** (2.3)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

6-MONTH STUDY IN Tg.AC HEMIZYGOUS MICE

Survival

Estimates of 6-month survival probabilities for male and female mice are shown in Table 10 and in the Kaplan-Meier survival curves (Figure 3). Survival of all dosed groups of mice was similar to that of the vehicle controls.

TABLE 10
Survival of Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
Animals initially in study	15	15	15	15	15	15
Natural deaths	1	0	3	1	2	4
Animals surviving to study termination	14	15	12	14	13	11
Percent probability of survival at end of study[a]	93	100	80	93	87	73
Mean survival (days)[b]	186	192	175	188	185	187
Survival analysis[c]	P=0.111	P=1.000N	P=0.567	P=1.000N	P=0.984	P=0.357
Female						
Animals initially in study	15	15	15	15	15	15
Moribund	0	0	1	0	0	0
Natural deaths	0	1	2	1	1	3
Animals surviving to study termination	15	14	12	14	14	12
Percent probability of survival at end of study	100	93	80	93	93	80
Mean survival (days)	193	192	171	188	191	187
Survival analysis[c]	P=0.305	P=1.000	P=0.226	P=1.000	P=1.000	P=0.224

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dosed group is indicated by **N**.

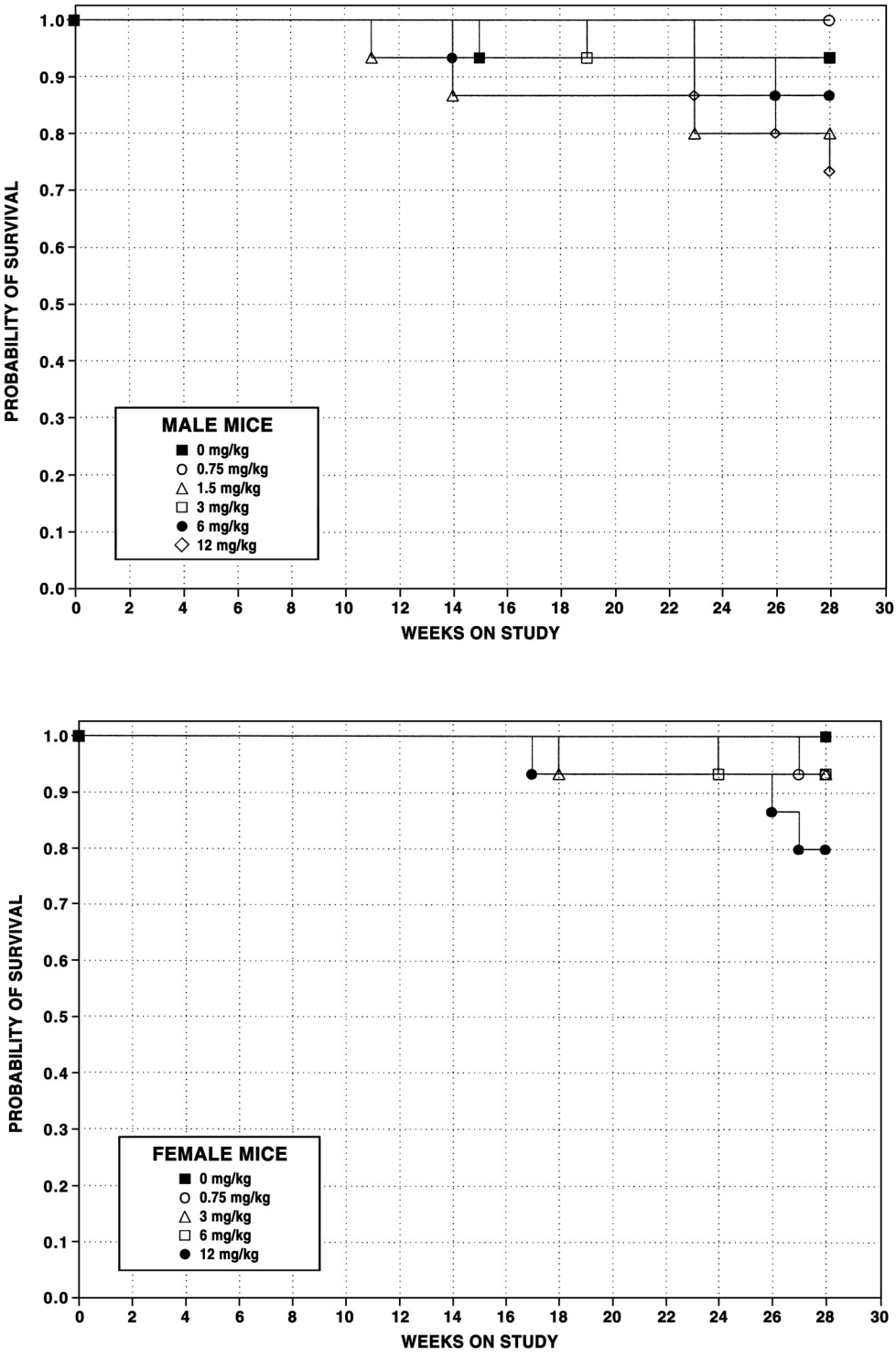


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Tg.AC Hemizgyous Mice Administered Trimethylolpropane Triacrylate Dermally for 6 Months

Body Weights and Clinical Findings

Mean body weights of dosed groups of mice were 95% to 105% those of the vehicle controls for most of the study (Tables 11 and 12; Figure 4). Treatment-related clinical findings included papillomas at the site of application in 3 mg/kg and greater males and 6 and 12 mg/kg females; one female administered 1.5 mg/kg also had a papilloma. In-life observations of papillomas at the site of application are summarized in Table 13. The number of mice with papillomas, the total number of papillomas, and the mean number of papillomas per mouse increased with increasing dose. Papillomas were observed earlier in the 6 and 12 mg/kg groups than in the other dose groups.

Organ Weights and Organ-Weight-to-Body-Weight Ratios

Liver weights were increased in 12 mg/kg male and female mice (Table F5). Absolute and relative lung weights of 6 and 12 mg/kg males and 12 mg/kg females were significantly less than those of the vehicle controls; absolute lung weights were also decreased in 6 mg/kg females. Heart weights of 12 mg/kg males and females (absolute only) were significantly increased. Females administered 12 mg/kg also had significantly increased kidney weights.

TABLE 11
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

Weeks on Study	Vehicle Control		0.75 mg/kg			1.5 mg/kg			3 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.1	15	21.9	99	15	21.3	96	15	21.9	99	15
2	22.7	15	22.5	99	15	22.5	99	15	22.9	101	15
3	24.3	15	23.3	96	15	24.0	99	15	23.9	98	15
4	25.3	15	24.5	97	15	24.8	98	15	25.1	99	15
5	25.9	15	25.2	97	15	26.2	101	15	26.4	102	15
6	27.2	15	26.3	97	15	27.5	101	15	26.6	98	15
7	27.3	15	26.8	98	15	27.9	102	15	27.4	100	15
8	28.2	15	27.5	98	15	28.5	101	15	28.1	100	15
9	28.0	15	26.7	95	15	28.7	103	15	28.5	102	15
10	28.6	15	27.1	95	15	29.1	102	15	28.6	100	15
11	29.2	15	27.9	96	15	29.5	101	15	29.0	99	15
12	29.7	15	28.3	95	15	30.6	103	14	29.2	98	15
13	29.3	15	28.7	98	15	30.3	103	14	29.7	101	15
14	30.0	15	29.2	97	15	29.9	100	14	30.3	101	15
15	30.4	15	28.6	94	15	31.7	104	13	31.1	102	15
16	30.3	14	29.3	97	15	31.4	104	13	31.0	102	15
17	30.1	14	29.9	99	15	31.7	105	13	31.7	105	15
18	31.3	14	30.4	97	15	31.8	102	13	32.0	102	15
19	31.4	14	30.1	96	15	32.3	103	13	32.2	103	15
20	31.3	14	30.9	99	15	31.2	100	13	32.7	105	14
21	31.2	14	30.2	97	15	32.6	105	13	32.5	104	14
22	31.9	14	31.2	98	15	33.2	104	13	33.4	105	14
23	32.1	14	31.1	97	15	32.6	102	13	32.9	103	14
24	31.7	14	31.9	101	15	33.9	107	12	33.8	107	14
25	33.1	14	32.0	97	15	35.3	107	12	34.3	104	14
26	32.5	14	32.1	99	15	33.8	104	12	34.3	106	14
27	33.1	14	32.2	97	15	35.2	106	12	34.5	104	14
28	32.6	14	32.5	100	15	36.3	111	12	33.8	104	14
Mean for weeks											
1-13	26.8		25.9	97		27.0	101		26.7	100	
14-28	31.5		30.8	98		32.9	104		32.7	104	

TABLE 11
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

Weeks on Study	6 mg/kg			12 mg/kg		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	21.7	98	15	21.0	95	15
2	21.2	93	15	22.7	100	15
3	23.0	95	15	23.9	98	15
4	24.9	98	15	24.8	98	15
5	25.9	100	15	26.2	101	15
6	27.3	100	15	26.7	98	15
7	27.6	101	15	27.2	100	15
8	27.9	99	15	28.1	100	15
9	27.9	100	15	28.0	100	15
10	28.3	99	15	28.2	99	15
11	28.8	99	15	28.5	98	15
12	28.8	97	15	28.9	97	15
13	28.7	98	15	29.1	99	15
14	29.3	98	14	29.6	99	15
15	30.2	99	14	30.0	99	15
16	29.8	98	14	30.1	99	15
17	30.3	101	14	30.5	101	15
18	31.3	100	14	30.7	98	15
19	30.5	97	14	31.3	100	15
20	30.6	98	14	31.9	102	15
21	30.8	99	14	31.7	102	15
22	31.2	98	14	32.0	100	15
23	31.0	97	14	32.2	100	15
24	31.9	101	14	32.9	104	13
25	30.8	93	14	32.9	99	13
26	31.6	97	14	33.1	102	13
27	32.4	98	13	33.5	101	12
28	32.8	101	13	33.0	101	12
Mean for weeks						
1-13	26.3	98		26.4	99	
14-28	31.0	98		31.7	100	

TABLE 12
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

Weeks on Study	Vehicle Control		0.75 mg/kg			1.5 mg/kg			3 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.6	15	18.4	99	15	18.5	100	15	18.6	100	15
2	19.3	15	19.0	98	15	19.4	101	15	18.6	96	15
3	20.4	15	20.9	103	15	20.9	103	15	20.7	102	15
4	21.7	15	21.6	100	15	21.8	101	15	21.2	98	15
5	22.8	15	22.7	100	15	22.8	100	15	22.8	100	15
6	23.3	15	23.1	99	15	23.6	101	15	23.6	101	15
7	23.8	15	23.7	100	15	23.3	98	15	23.6	99	15
8	22.8	15	23.6	104	15	23.6	104	15	24.5	108	15
9	24.1	15	23.4	97	15	23.5	98	15	23.0	95	15
10	24.3	15	24.6	101	15	25.0	103	13	24.3	100	15
11	24.4	15	25.2	103	15	25.3	104	13	25.0	103	15
12	24.6	15	25.2	102	15	25.6	104	13	24.8	101	15
13	25.1	15	25.5	102	15	25.6	102	13	25.6	102	15
14	24.7	15	25.8	105	15	25.7	104	13	25.3	102	15
15	26.2	15	25.8	99	15	26.9	103	13	26.0	99	15
16	25.7	15	25.8	100	15	26.0	101	13	26.0	101	15
17	26.1	15	26.4	101	15	26.9	103	13	26.3	101	15
18	26.1	15	26.6	102	15	26.7	102	13	26.1	100	15
19	25.5	15	26.2	103	15	26.5	104	12	26.3	103	14
20	26.0	15	26.6	102	15	27.3	105	12	27.3	105	14
21	26.3	15	26.8	102	15	27.6	105	12	27.0	103	14
22	27.0	15	26.7	99	15	27.4	102	12	26.9	100	14
23	27.2	15	26.7	98	15	26.9	99	12	27.5	101	14
24	27.5	15	26.9	98	15	28.4	103	12	27.6	100	14
25	27.5	15	26.9	98	15	28.4	103	12	27.6	100	14
26	27.1	15	27.3	101	15	28.1	104	12	26.6	98	14
27	27.9	15	27.5	99	14	27.4	98	12	27.9	100	14
28	28.2	15	27.6	98	14	28.9	103	12	28.5	101	14
Mean for weeks											
1-13	22.7		22.8	101		23.0	101		22.8	100	
14-28	26.6		26.6	100		27.3	103		26.9	101	

TABLE 12
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

Weeks on Study	6 mg/kg			12 mg/kg		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.7	101	15	18.5	100	15
2	18.7	97	15	19.4	101	15
3	20.1	99	15	20.2	99	15
4	21.5	99	15	21.7	100	15
5	22.3	98	15	22.3	98	15
6	23.4	100	15	22.5	97	15
7	23.0	97	15	23.2	98	15
8	24.0	105	15	23.3	102	15
9	23.7	98	15	23.8	99	15
10	24.5	101	15	23.2	96	15
11	24.9	102	15	24.5	100	15
12	25.0	102	15	24.8	101	15
13	24.8	99	15	25.2	100	15
14	25.4	103	15	25.3	102	15
15	26.0	99	15	25.5	97	15
16	25.7	100	15	24.6	96	15
17	26.6	102	15	26.2	100	15
18	26.2	100	15	25.8	99	14
19	26.3	103	15	26.8	105	14
20	27.2	105	15	27.4	105	14
21	27.1	103	15	27.2	103	14
22	26.3	97	15	27.5	102	14
23	26.4	97	15	27.4	101	14
24	27.8	101	14	28.2	103	14
25	27.6	100	14	27.6	100	14
26	27.3	101	14	28.4	105	13
27	26.4	95	14	28.7	103	13
28	27.9	99	14	28.7	102	12
Mean for weeks						
1-13	22.7	100		22.5	99	
14-28	26.7	100		27.0	102	

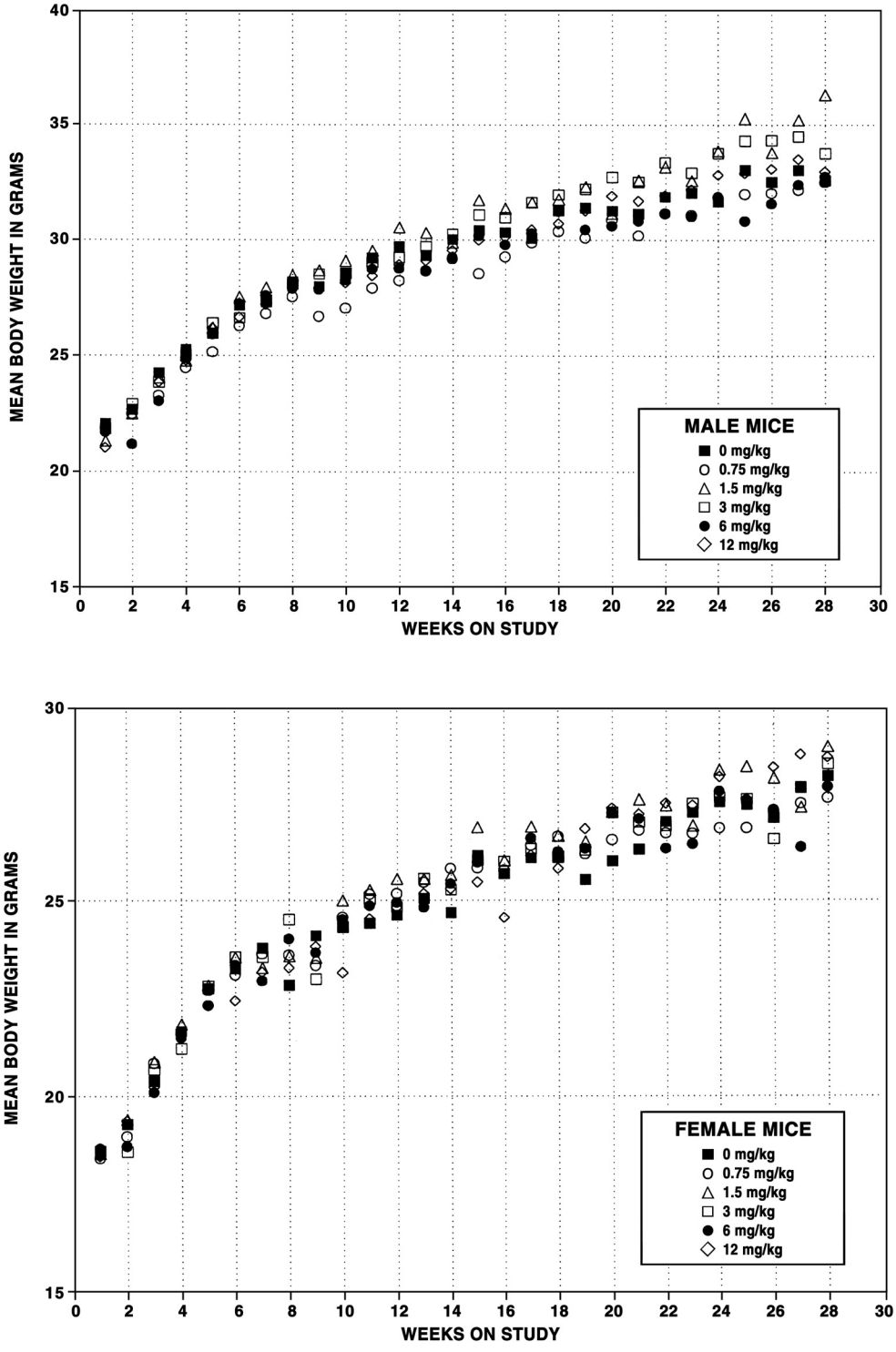


FIGURE 4
Growth Curves for Male and Female Tg.AC Hemizygous Mice Administered Trimethylolpropane Triacrylate Dermally for 6 Months

TABLE 13
Skin Papilloma Formation at the Site of Application in Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate[a]

Dose (mg/kg)	Number (Percent) with Papilloma[b]		Time to Initial Papilloma Occurrence for All Animals in Group[c] (Week)		Distribution of Number of Papillomas per Animal[d] Quantiles			Test of Dunson *et al.* Model[e]	
			First	Median	20th	50th	80th	γ_1	γ_2
Male									
0	0	(0.0%)	NA	>28	0	0	0		
0.75	0	(0.0%)	NA	>28	0	0	0		NT
1.5	0	(0.0%)	NA	>28	0	0	0		NT
3	3	(20.9%)	23	>28	0	0	0		NT
6	13	(91.9%)	14	20	1	6	15.5	**	NT
12	13	(86.7%)	9	13	20+	20+	20+	**	NT
Trend								**	**
Positive Control[f]	15	(100.0%)	9	10	9	20+	20+	**	NT
Female									
0	0	(0.0%)	NA	>28	0	0	0		
0.75	0	(0.0%)	NA	>28	0	0	0		NT
1.5	1	(8.1%)	19	>28	0	0	0		NT
3	2	(14.0%)	28	>28	0	0	0		NT
6	12	(80.0%)	9	25	0	1	4	**	NT
12	15	(100.0%)	10	14	20+	20+	20+	**	NT
Trend								**	**
Positive Control	14	(99.8%)	9	10	1	16	20+	**	NT

[a] 15 males and 15 females initially in each dose group
[b] Percent is Poly-3 adjusted rate and reflects whether the animal ever had a confirmed papilloma at any point in the study.
[c] For groups in which fewer than half of the animals had papillomas, the median time to initial occurrence is >28 weeks. NA=not applicable.
[d] Quantiles are based on all animals in a group, whether removed before the end of study or not. For example, a value of 9 for the 20th quantile implies that 20% of the animals in the study had 9 papillomas or fewer at the end of the study (or at removal from study).
[e] The Dunson *et al.* (2000) model accounts for latency (γ_1) and multiplicity (γ_2) in the rate of occurrence, NT (No Test) indicates that a pairwise test could not be meaningfully applied because no papillomas were observed in the control group. ** ($P \leq 0.01$) indicates a significant trend or a significant difference from the vehicle control group.
[f] 100 µL of 1.25 µg 12-*O*-tetradecanoylphorbol-13-acetate per 100 mL acetone administered three times per week.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the skin determined by microscopic evaluation; forestomach; liver; spleen; and mandibular, mediastinal, and mesenteric lymph nodes. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male mice and Appendix B for female mice.

Skin: Squamous cell neoplasms at the site of application were associated with dermal application of trimethylolpropane triacrylate. The incidences of squamous cell papilloma were significantly increased in 6 and 12 mg/kg males and females (Tables 14, A3, and B3). Papillomas were multiple in all 12 mg/kg males and females and 6 mg/kg males. Two males and one female in the 3 mg/kg groups also had squamous cell papillomas at the site of application. Squamous cell papilloma had the typical morphology of an exophytic growth of well-differentiated squamous epithelium covering arborizing fronds of connective tissue. Multiple papillomas were often contiguous. One female in each of the 1.5, 6, and 12 mg/kg groups also had a squamous cell carcinoma at the site of application (Plate 8). These carcinomas appeared to arise within papillomas (Plate 9), with extensions of atypical squamous cells into the underlying dermis and subcutis. Squamous cell carcinomas at the site of application were considered to be treatment-related and possibly the result of malignant conversion of papillomas.

Nonneoplastic effects at the site of application were squamous cell hyperplasia, hyperkeratosis, and chronic active inflammation. Hyperplasia was present in the 3 mg/kg and greater groups and was characterized by increased thickness of the epidermis, from the normal one to three cell layers to four to six cell layers thick (minimal to mild severity). The hyperplasia was generally diffuse in areas between papillomas. Occasionally a focal nodular type of hyperplasia was observed that was considered a precursor lesion to squamous papilloma. Hyperplasia was accompanied by minimal to mild increased thickness of the keratin layer (hyperkeratosis). A minimal to mild infiltrate of mixed inflammatory cells (chronic active inflammation) was present in the dermis, generally in the 6 and 12 mg/kg groups. Some inflammation was invariably associated with neoplasms, but the diagnosis was made when infiltrates were observed in nonneoplastic areas. Sebaceous glands at the site of application were enlarged (hyperplasia) in several males and females administered 3 mg/kg or greater, but this lesion was not diagnosed separately.

TABLE 14
Incidences of Neoplasms and Nonneoplastic Lesions of the Skin (Site of Application) in Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
Number Examined						
Microscopically	15	15	15	15	15	15
Epidermis, Hyperplasia[a]	0	0	0	6** (1.0)[b]	14** (1.7)	15** (1.9)
Hyperkeratosis	0	0	1 (2.0)	15** (1.0)	14** (1.1)	12** (1.7)
Inflammation, Chronic Active	0	0	1 (1.0)	1 (1.0)	9** (1.1)	12** (1.3)
Squamous Cell Papilloma, Multiple	0	0	0	0	12**	13**
Squamous Cell Papilloma (includes multiple)						
Overall rate[c]	0/15 (0%)	0/15 (0%)	0/15 (0%)	2/15 (13%)	12/15 (80%)	13/15 (87%)
Adjusted rate[d]	0.0%	0.0%	0.0%	14.0%	85.1%	86.7%
Terminal rate[e]	0/14 (0%)	0/15 (0%)	0/12 (0%)	2/14 (14%)	11/13 (85%)	9/11 (82%)
First incidence (days)	—[g]	—	—	192 (T)	180	161
Poly-3 test[f]	P<0.001	—[h]	—	P=0.234	P<0.001	P<0.001
Female						
Number Examined						
Microscopically	15	15	15	15	15	15
Epidermis, Hyperplasia	0	0	1 (2.0)	4* (1.0)	15** (1.2)	15** (2.0)
Hyperkeratosis	0	0	1 (1.0)	7** (1.0)	14** (1.1)	13** (1.6)
Inflammation, Chronic Active	0	0	0	3 (1.0)	14** (1.0)	12** (1.3)
Squamous Cell Papilloma, Multiple	0	0	0	0	5*	15**
Squamous Cell Papilloma (includes multiple)						
Overall rate	0/15 (0%)	0/15 (0%)	0/15 (0%)	1/15 (7%)	11/15 (73%)	15/15 (100%)
Adjusted rate	0.0%	0.0%	0.0%	7.0%	73.3%	100%
Terminal rate	0/15 (0%)	0/14 (0%)	0/12 (0%)	1/14	10/14 (71%)	12/12 (100%)
First incidence (days)	—	—	—	193 (T)	162	118
Poly-3 test	P<0.001	—	—	P=0.490	P<0.001	P<0.001
Squamous Cell Carcinoma	0	0	1	0	1	1

(T) Terminal sacrifice
* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Number of neoplasm-bearing animals/number of animals with skin examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence is the P value corresponding to pairwise comparison between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[g] Not applicable; no neoplasms in animal group
[h] Value of statistic cannot be computed.

Forestomach: The incidence of squamous cell papilloma in 12 mg/kg females was significantly greater than that in the vehicle control group (Tables 15 and B3). The morphology of these neoplasms was similar to that of skin papillomas. Multiple papillomas occurred in three of the nine affected females in the 12 mg/kg group, in contrast to single incidences in all other groups. No other proliferative squamous lesions (hyperplasia or carcinoma) were observed. Forestomach papilloma is a relatively common spontaneous finding in Tg.AC mice that may occur at a high and variable rate (10%-25% in hemizygous females; higher in homozygous mice; Mahler *et al.*, 1998). The increased incidence of forestomach papilloma in 12 mg/kg females may have been treatment related.

TABLE 15
Incidences of Squamous Cell Papilloma of the Forestomach in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Number Examined Microscopically	15	15	15	15	15	15
Squamous Cell Papilloma, Multiple[a]	1	1	1	1	1	3
Squamous Cell Papilloma (includes multiple)						
Overall rate[b]	4/15 (27%)	5/15 (33%)	4/15 (27%)	2/15 (13%)	5/15 (33%)	9/15 (60%)
Adjusted rate[c]	26.7%	33.7%	32.5%	14.0%	34.3%	64.6%
Terminal rate[d]	4/15 (27%)	5/14 (36%)	4/12 (33%)	2/14 (14%)	5/14 (36%)	9/12 (75%)
First incidence (days)	193 (T)	193 (T)	193 (T)	193 (T)	193 (T)	193 (T)
Poly-3 test[e]	P=0.014	P=0.493	P=0.535	P=0.352N	P=0.481	P=0.040

(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Number of neoplasm-bearing animals/number of animals with forestomach examined microscopically
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence is the P value corresponding to pairwise comparison between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by **N**.

Other Organs: Incidences of hematopoietic cell proliferation in various tissues were increased in dosed mice (Tables 16, A4, and B4). Hematopoietic cell proliferation was significantly increased in the liver of 12 mg/kg males and females and the spleen of 6 and 12 mg/kg males, as well as in the mandibular, mediastinal, and mesenteric lymph node of 12 mg/kg females. Hematopoietic cell proliferation consisted of increased numbers of erythroid and granulocytic precursors in the splenic red pulp, liver sinusoids, or nodal parenchyma. These changes were attributed to the dermal inflammatory stimulus at the application site and/or release of hematopoietic cytokines and growth factors from proliferative epidermal cells.

There was a change that occurred in some animals administered 6 or 12 mg/kg. This change was observed in one or more organs and was characterized by somewhat variable morphology and uncertain biological behavior. Florid lesions, diagnosed as myelodysplasia, were identified in two males and two females exposed to 12 mg/kg, while milder lesions, diagnosed as cell, infiltration, nonspecified site, were identified in several animals (Tables 16, A4, and B4). The change was characterized predominantly by myeloid infiltration/proliferation that tended to be perivascular in the liver and lungs. Infiltrating cells were predominantly mature and immature granulocytes (eosinophils and neutrophils) with lesser numbers of admixed mononuclear cells. In severely affected livers, there was bridging between portal tracts and accumulations of brightly eosinophilic crystalline material in the lumen of bile ductules. The change was commonly observed in the mediastinal, mandibular, axillary, and mesenteric lymph nodes, and often included a pronounced plasma cell population in the medullary sinuses. The epididymis and spleen were also often involved.

TABLE 16
Incidences of Selected Nonneoplastic Lesions in Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control		0.75 mg/kg		1.5 mg/kg		3 mg/kg		6 mg/kg		12 mg/kg	
Male												
Liver[a]	15		15		15		15		15		15	
Hematopoietic Cell Proliferation[b]	1	(1.0)[c]	0		1	(1.0)	0		0		6*	(1.3)
Spleen	15		15		15		15		15		15	
Hematopoietic Cell Proliferation	1	(2.0)	4	(1.5)	1	(3.0)	2	(1.5)	6*	(2.0)	8**	(2.6)
All Organs[d]	15		15		15		15		15		15	
Myelodysplasia	0		0		0		0		0		2	
Infiltration Cellular	0		0		0		0		0		5*	
Infiltration Cellular, Plasma Cell	0		0		0		0		0		4*	
Female												
Liver	15		15		15		15		15		15	
Hematopoietic Cell Proliferation	0		0		1	(2.0)	0		0		6**	(1.8)
Lymph Node, Mandibular	15		15		15		15		15		15	
Hematopoietic Cell Proliferation	0		0		1	(3.0)	0		0		6**	(2.0)
Lymph Node, Mediastinal	11		14		13		10		13		12	
Hematopoietic Cell Proliferation	0		0		0		0		0		5*	(2.0)
Lymph Node, Mesenteric	15		14		14		15		15		15	
Hematopoietic Cell Proliferation	0		0		0		0		0		4*	(2.0)
All Organs	15		15		15		15		15		15	
Myelodysplasia	0		0		0		0		0		2	
Infiltration Cellular	0		0		1		0		0		3	
Infiltration Cellular, Plasma Cell	0		0		1		0		0		2	

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Number of animals with any tissue examined microscopically

Contact Hypersensitivity Studies in BALB/c Mice

These studies are described in Appendix K. There were no deaths, body weight changes, or clinical findings related to trimethylolpropane triacrylate treatment in dosed mice. Results of the irritancy study indicated that the maximal nonirritating and minimal irritating doses were 0.1% and 0.25% trimethylolpropane triacrylate, respectively (Figure K4).

In the mouse ear swelling test, no significant differences in the percentage of ear swelling were observed between the trimethylolpropane triacrylate-sensitized and challenged mice and the background controls at 24 or 48 hours after dosing (Figure K5). The local lymph node assay indicated no significant increase in lymph node cell proliferation in mice administered trimethylolpropane triacrylate compared to that in the vehicle controls (Figure K6).

Testing for sensitizing potential using the mouse ear swelling test and local lymph node assay failed to indicate trimethylolpropane triacrylate as a potential contact sensitizer at the concentrations tested.

Genetic Toxicology

No increase in the frequency of micronucleated normochromatic erythrocytes was observed in peripheral blood samples from male or female mice administered dermal applications of 0.75 to 12 mg trimethylolpropane triacrylate/kg body weight for 3 (Table C1) or 6 months (Table C2). In the 3-month study, ratios of micronucleated polychromatic erythrocytes to NCEs in peripheral blood were unaltered by chemical treatment, indicating an absence of induced bone marrow toxicity. However, in the 6-month study, decreases in the percentages of circulating NCEs among total erythrocytes were noted in 12 mg/kg male and female mice, indicating a stimulation of erythropoiesis and the presence of increased numbers of immature erythrocytes in circulating blood.

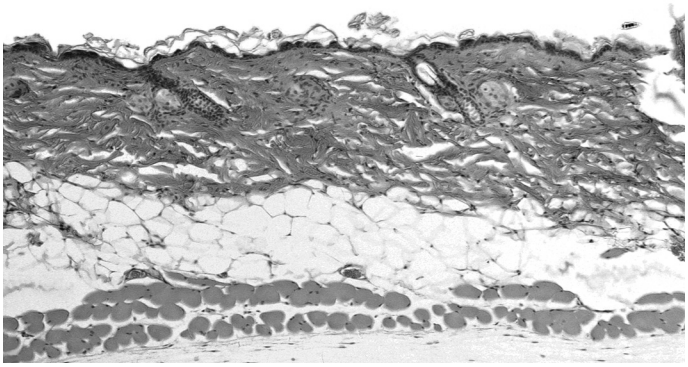


PLATE 1
Normal skin (site of application) from a vehicle control B6C3F$_1$ mouse in the 3-month dermal study. H&E; 25×

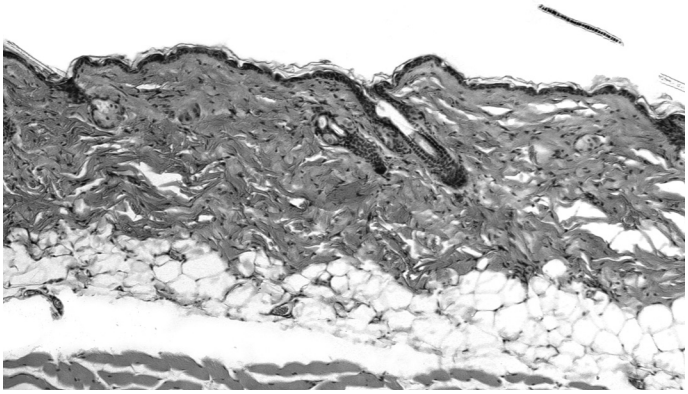


PLATE 2
Skin (site of application) from a male B6C3F$_1$ mouse treated with 0.75 mg/kg trimethylolpropane triacrylate in the 3-month dermal study. There is no discernible difference from the vehicle control mouse skin in Plate 1. H&E; 25×

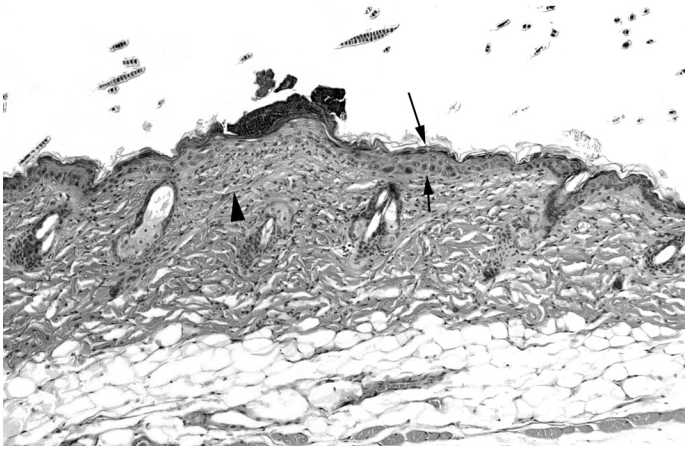


PLATE 3
Skin (site of application) from a male B6C3F$_1$ mouse treated with 1.5 mg/kg trimethylolpropane triacrylate in the 3-month dermal study. When compared to the vehicle control mouse skin (Plate 1), there is an increased thickness (hyperplasia) of the epidermis (arrows) and increased cellularity (inflammation) of the superficial dermis (arrowheads). H&E; 25×

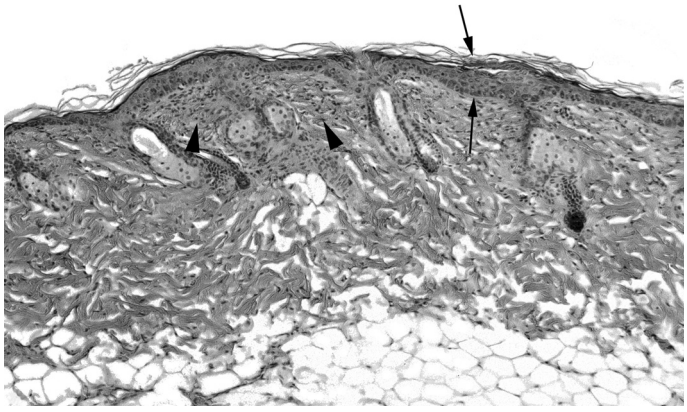


PLATE 4
Skin (site of application) from a male B6C3F$_1$ mouse treated with 3 mg/kg trimethylolpropane triacrylate in the 3-month dermal study. Note the patchy hyperkeratosis and hyperplasia (arrows) of the epidermis and inflammation of the dermis (arrowheads). H&E; 25×

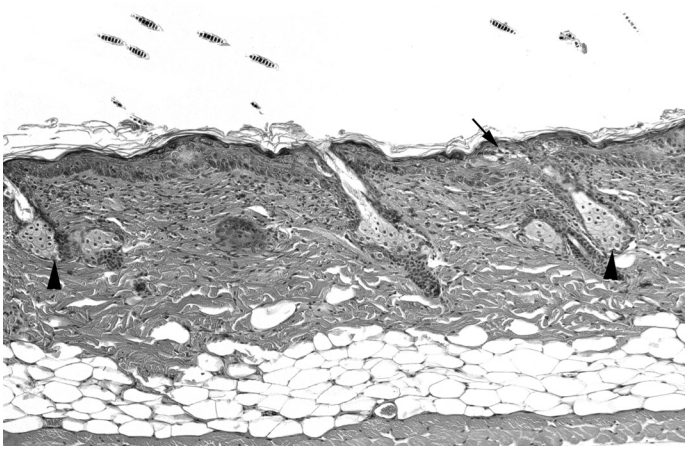


PLATE 5
Skin (site of application) from a male B6C3F$_1$ mouse treated with 6 mg/kg trimethylolpropane triacrylate in the 3-month dermal study. The epidermis is hyperplastic and focally disrupted [degeneration (arrow)]. The dermis is inflamed and sebaceous glands are large (hyperplasia) and prominent (arrowheads). H&E; 25×

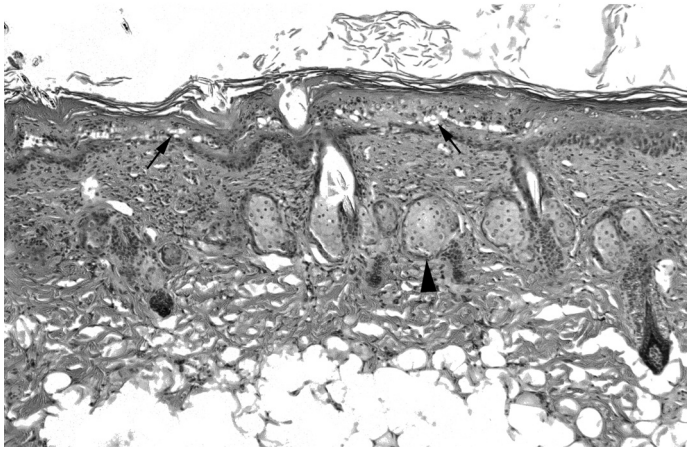


PLATE 6
Skin (site of application) from a male B6C3F$_1$ mouse treated with 12 mg/kg trimethylolpropane triacrylate in the 3-month dermal study. Diffuse hyperkeratosis, hyperplasia, and degeneration (arrows) of the epidermis. The hyperplastic sebaceous glands are also very prominent (arrowheads). H&E; 25×

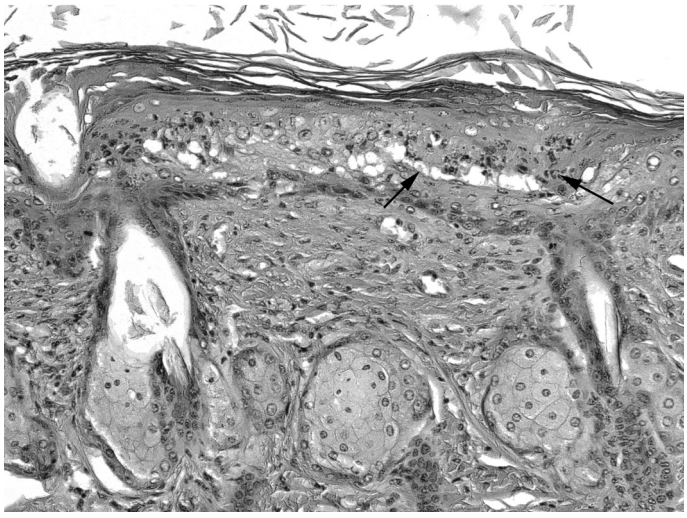


PLATE 7
Skin (site of application) from a male B6C3F$_1$ mouse treated with 12 mg/kg trimethylolpropane triacrylate in the 3-month dermal study. Higher magnification of Plate 6 to illustrate the prominent epidermal degeneration, necrosis (arrows), and inflammation. Also note the dermal inflammation and sebaceous gland hyperplasia. H&E; 50×

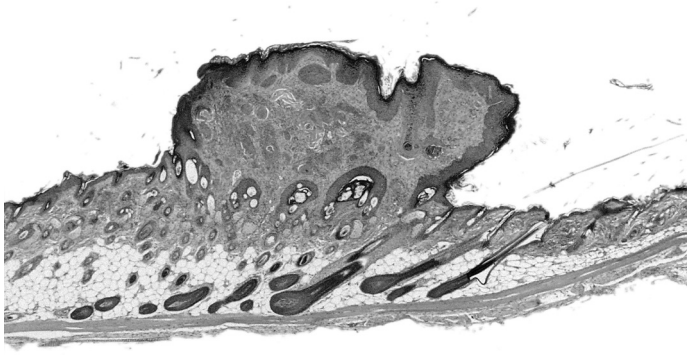


PLATE 8
Skin from a female Tg.AC mouse treated with 12 mg/kg trimethylopropane triacrylate in the 6-month dermal study. This is an early squamous cell carcinoma that appears to be arising within a squamous cell papilloma. H&E; 2×

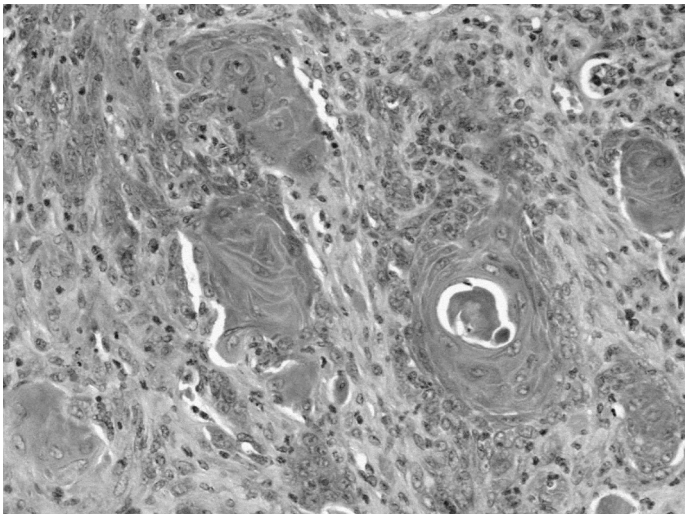


PLATE 9
Higher magnification of Plate 8. Note the islands of squamous epithelium within the base of the papillomatous mass. H&E; 20×

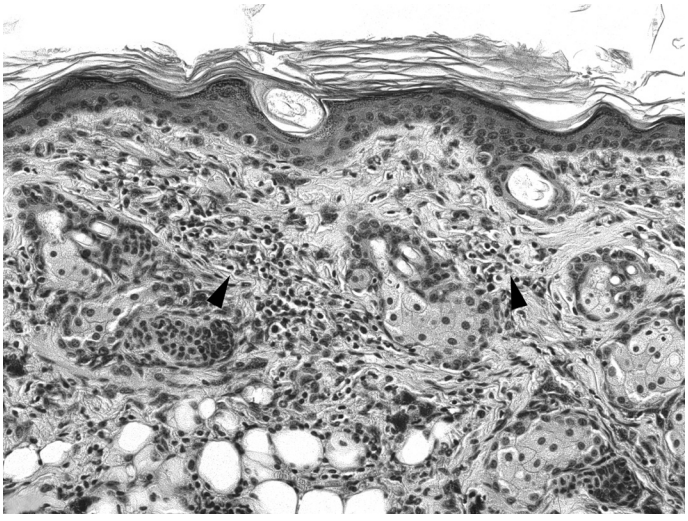


PLATE 10
Skin (site of application) in a Tg.AC mouse treated with 166 mg/kg rotenone in the 24-week skin application study. The magnification is the same as in Plate 7. Note the intense inflammatory infiltrate (arrowheads) in the dermis and epidermal hyperplasia. H&E; 50×

DISCUSSION AND CONCLUSIONS

Trimethylolpropane triacrylate and pentaerythritol triacrylate are representative multifunctional acrylates. Chemically, multifunctional acrylates are esters of acrylic acid esterified to a polyhydroxy backbone molecule. Trimethylolpropane triacrylate is a triester of acrylic acid with trimethylol propane, and pentaerythritol triacrylate is a triester of acrylic acid with pentaerythritol.

Monofunctional acrylates, such as ethyl acrylate, and multifunctional acrylates, such as trimethylolpropane triacrylate and pentaerythritol triacrylate, readily undergo free radical polymerization initiated by a peroxy compound or by ultraviolet light. They are therefore used in photocurable inks and in other applications requiring the use of photocurable resins. Multifunctional acrylates are also used in glues and adhesives and in the manufacture of acrylic-based paints. The NTP studied trimethylolpropane triacrylate and pentaerythritol triacrylate for toxicity and carcinogenicity. Results of the pentaerythritol triacrylate studies are reported separately (NTP, 2005).

Human exposure to multifunctional acrylates has been documented only in occupational settings and has involved primarily dermal exposure (NIOSH, 1990). Workers involved in the manufacture, processing, product handling, and application of trimethylolpropane triacrylate are at risk of exposure (AIHA, 1981). However, the widespread use of these compounds in the manufacture of consumer products such as certain glues, adhesives, and paints suggests a potential for significant nonoccupational exposure (Dearfield *et al.*, 1989).

From the available data in the literature, the critical effects of multifunctional acrylates are skin and eye irritation. Also, some members of the multifunctional acrylate class are moderate to strong sensitizers in humans, but findings in animals are conflicting. Trimethylolpropane triacrylate was not detected as a sensitizer in repeated-insult patch testing or in guinea pigs using the Buehler method. However, trimethylolpropane triacrylate was positive in a guinea pig maximization test (Andrews and Clary, 1986). The NTP has tested trimethylolpropane triacrylate for its ability to induce hypersensitivity and irritancy in a rodent model (Appendix K). Trimethylolpropane triacrylate was found to be an irritant in BALB/c mice at concentrations greater than 0.25%. There was no significant difference compared to the vehicle controls at any single dose in the local lymph node assay. Results of the mouse ear-swelling test were negative at both 24 and 48 hours after dosing for the trimethylolpropane triacrylate concentrations studied. Overall, the studies in BALB/c mice suggest that trimethylolpropane triacrylate is an irritant but not a contact sensitizer.

Repeated dermal exposure to acrylates including trimethylolpropane triacrylate led to contact dermatitis in laboratory animals and humans (Andrews and Clary, 1986); the current 2-week and 3-month studies confirmed these findings. Skin was the major target organ of trimethylolpropane triacrylate toxicity in the current studies. The chemical caused dose-related hyperplastic, vacuolar degenerative, and ultimately necrotizing lesions of the epidermis, accompanied by hyperplasia of sebaceous glands and a chronic dermal inflammation in both sexes of rats and mice. Rats had slightly more severe skin lesions than mice in the 2-week and 3-month studies. Although trimethylolpropane triacrylate is absorbed through skin as shown by additional NTP studies (Appendix L), systemic toxicity was minimal, based on clinical pathology, histopathology, and organ weight findings. In rats, a no-observed-adverse-effect level (NOAEL) was not attained; the NOAEL was determined to be 0.75 mg/kg in mice.

The dose-related skin effects such as inflammation and sustained hyperplasia at the site of application in rats and mice in the 3-month studies suggested trimethylolpropane triacrylate may be a dermal carcinogen. Several studies have established that the induction of sustained cellular hyperplasia correlates well with the skin tumor formation ability of various tumor-promoting agents such as phorbol esters, several peroxides, and chrysarobin (Argyris, 1981; Hennings *et al.*, 1993; Slaga

et al., 1995). NTP typically performs carcinogenicity studies in two species, rats and mice. Because skin was the only major target organ of toxicity in the 2-week and 3-month studies, dermal carcinogenicity studies were conducted in mice only. For more than 60 years, studies have established the mouse as a sensitive animal model for epidermal carcinogenesis induced by chemical carcinogens (DiGiovanni, 1991). In addition, the mouse is also thought to be an appropriate model for skin squamous cell cancer development in humans because of evidence that known genetic aberrations in tumor cells are similar in human and mouse skin, especially mutational activation of the H-*ras* oncogene (Nagase *et al.*, 1996).

Leder *et al.* (1990) developed a transgenic mouse model with an inducible *zeta*-globin promoter driving the expression of a mutated v-H-*ras* oncogene (the Tg.AC mouse). With the exception of the bone marrow, constitutive expression of the transgene cannot be detected in adult tissues. The transgene is transcriptionally silent until activated by full-thickness wounding, ultraviolet light, or specific chemical exposure (Cannon *et al.*, 1997; Trempus *et al.*, 1998). Topical application of carcinogens to the shaved dorsal surface of Tg.AC mice induces epidermal squamous cell papillomas or carcinomas, a reporter phenotype that defines the activity of the chemical. The oral route of administration can also generate tumorigenic responses in Tg.AC mice and result in squamous cell papillomas or carcinomas of the forestomach. To date, the induction of either spontaneous or induced tumors has been shown to require activation of transgene expression. Thus, the Tg.AC model could be viewed as genetically initiated due to the presence of the transgene. The model responds to both genotoxic and nongenotoxic carcinogens (Spalding *et al.*, 1999, 2000; Tennant *et al.*, 2001).

When the current 3-month studies in rats and mice were completed, the NTP was evaluating several transgenic mouse models to supplement or replace the traditional 2-year bioassay studies in mice. The major route of human exposure to trimethylolpropane triacrylate is via the skin, and at the time of study design, the Tg.AC mouse model was showing promise for carcinogenicity testing (hazard identification) via dermal exposure. Efforts were under way to more fully assess the model's potential. Therefore, the NTP decided to conduct initial studies in the Tg.AC model, and upon completion of these studies, assess their findings in light of updated information about the Tg.AC model. Even if testing in additional species or strains were necessary, the Tg.AC studies would add to the body of knowledge concerning the Tg.AC model's potential. This allowed assessment of the Tg.AC model in a completely prospective manner. The Tg.AC mouse model's usefulness and limitations in detecting chemical carcinogens have since been evaluated (Spalding *et al.*, 2000; Eastin *et al.*, 2001; Tennant *et al.*, 2001; Pritchard *et al.*, 2003; Sistare *et al.*, 2002).

For the current 6-month dermal study in Tg.AC mice, the selected doses were based on the results of the 2-week and 3-month studies in F344/N rats and B6C3F$_1$ mice. Others have used 2- to 4-week studies in the Tg.AC mouse or FVB/N wildtype parent strain for dose range-finding studies (Tennant *et al.*, 1999; Eastin *et al.*, 2001). However, the dermal response to compounds was not expected to differ markedly between the mouse strains. While some information may have been lost by use of a different mouse strain, in this case, additional information concerning the effects of trimethylolpropane triacrylate on the skin was also gained by the use of two species and the conduct of a 3-month study rather than the typically shorter Tg.AC range-finding study.

When selecting doses for a 2-year dermal study based on 3-month study findings, dose concentrations that cause ulceration, necrosis, or marked inflammation or hyperplasia at the site of application are generally avoided. Therefore, because of the presence of mild necrosis and minimal to mild degeneration and suppurative inflammation, 12 mg/kg would not have been selected for use in a 2-year study. These pathologic alterations are consistent with the irritation identified grossly in the 12 mg/kg group in the 3-month study. Necrosis and degeneration occurred in fewer animals and/or with lesser severity in the 6 mg/kg group and hardly at all in lower dose groups. However, because doses were being selected for a different strain and for a 6-month rather than a 2-year study, five dose groups were used, and 12 mg/kg was the highest dose used.

In mouse dermal carcinogenicity studies, squamous cell papillomas are used as an endpoint of the assay. Some of these papillomas have the potential to progress to squamous cell carcinomas, so they may be regarded as precursor neoplasms and may be used as a quantitative indicator of a carcinogenic process (Enzmann *et al.*, 1998). The results of the current 6-month study clearly show the carcinogenic activity of trimethylolpropane triacrylate at the site of application in Tg.AC mice. The increased incidences of squamous cell papilloma were doserelated in males and females; and the incidences

were significantly increased in 6 and 12 mg/kg males and females. Squamous cell carcinomas occurred in a few female mice and their presence at the base of a papilloma in some mice suggested the carcinoma arose from the papilloma.

In the 6-month study, most animals in the 6 and 12 mg/kg groups had epidermal hyperplasia, hyperkeratosis, and chronic active inflammation at the site of application. The incidences of these nonneoplastic lesions were much lower in groups administered 3 mg/kg or less. Histologic evaluation of the skin for nonneoplastic lesions in the presence of multiple papillomas can be problematic, and there was often a significant amount of inflammation associated with the neoplasms. However, minimal to mild nonneoplastic lesions were also observed in the standard sections of skin at the site of application in which neoplasms were generally not present. Also, similar lesions as well as more severe lesions such as necrosis and degeneration were observed in $B6C3F_1$ mice at 3 months. Therefore the nonneoplastic lesions were likely primary and not secondary to the papillomas. In the 3-month study in $B6C3F_1$ mice, the more severe lesions (necrosis, degeneration, and suppurative inflammation) at the site of application occurred primarily at the same doses, 6 and 12 mg/kg, at which neoplasms occurred in Tg.AC mice. However, epidermal hyperplasia and chronic active inflammation, although more severe in the 6 and 12 mg/kg groups, also occurred in 3 mg/kg mice in the 3-month study.

An increase in cell replication enhances all steps of neoplastic transformation and tumor development (Enzmann *et al.*, 1998). In a study of resorcinol, increased incidences of neoplasms occurred in males and females concurrently with epidermal hyperplasia and in males concurrently with inflammation and sebaceous gland hyperplasia (NTP, 1992; Eastin *et al.*, 1998). However, in an NTP dermal carcinogenicity study on rotenone in Tg.AC mice, no skin papillomas were observed, despite extensive hyperplasia and inflammation in the skin at the site of application (Plate 10; Eastin *et al.*, 1998). This result suggests that the relationship between nonneoplastic lesions of the skin and papilloma induction is complex.

In some instances, such as with diethylstilbestrol, chemically induced skin neoplasms occurred in the absence of nonneoplastic effects (Eastin *et al.*, 1998). Dinitrofluorobenzene caused an increase in skin neoplasms in the Tg.AC mouse that was concluded to be associated with the cytotoxic action of the chemical to the skin (Albert *et al.*, 1996). The authors proposed that the response to dinitrofluorobenzene may be analogous to physical wounding, which promotes neoplasms in normal animals (Argyris, 1980), as well as in the Tg.AC mouse (Spalding *et al.*, 1993). Why chemical or physical wounding causes neoplasm promotion is not known, but may involve both cell damage and inflammation.

Insertion of the *zeta*-globin promoted v-Ha-*ras* transgene into the FVB mouse genome (Tg.AC) introduces a defined genetic lesion that is critical but insufficient by itself to induce benign or malignant tumors in skin unless activated. Activation and expression of the transgenic *ras* oncoprotein in this mouse line induces dose-related increases in papilloma (skin reporter phenotype) within weeks. In the current 6-month study, trimethylolpropane triacrylate induced dose-related increases in the incidence of squamous cell papilloma at the site of application, with neoplasms appearing as early as 9 weeks into the study. Some papillomas in female mice appeared to progress to carcinoma. Two other multifunctional acrylates, tripropylene glycol diacrylate (Nylander-French and French, 1998) and pentaerythritol triacrylate (NTP, 2005) also induced skin papillomas in this model, suggesting the model is responsive to multifunctional acrylates. The Tg.AC model appears to respond to both genotoxic and nongenotoxic carcinogens. Chemically induced sustained cell proliferation may be an important component of the multistage process of mouse skin carcinogenesis, particularly for nongenotoxic carcinogens.

In one previous study, no carcinogenic activity was noted in mice exposed to trimethylolpropane triacrylate. Dermal carcinogenicity studies on eight multifunctional acrylates including trimethylolpropane triacrylate were conducted by the Celanese Corporation in C3H/HeJ mice (Andrews and Clary, 1986). Trimethylolpropane triacrylate was administered to the intrascapular region of the skin at a dose level of 100 mg/kg in mineral oil twice weekly for 80 weeks. No skin tumors or systemic lesions were observed. The absence of carcinogenic activity in that study may be due to differences in study design and strain of mouse used. The dose level, 100 mg/kg, was more than eight times the current study's highest dose. No major effects on the skin were noted, most likely due to the use of mineral oil as a vehicle. Mineral oil is known to ameliorate the irritating properties of chemicals (Nessel *et al.*, 1999).

The only systemic neoplastic effect seen in the current 6-month trimethylolpropane triacrylate study was an increased incidence of squamous cell papilloma of the forestomach in 12 mg/kg female mice that exceeded the historical control values in Tg.AC mice (Mahler *et al.*, 1998). The incidence of forestomach squamous cell papilloma in 12 mg/kg females was significantly higher than that in the controls (60% and 27%, respectively) and well above reported historical control rates of 10% (Mahler *et al.*, 1998) and 25% (Eastin *et al.*, 2001). The background rate of forestomach neoplasms appears highly variable; rates of 40% to 75% have been reported in some dietary studies performed in homozygous Tg.AC mice (Sistare *et al.*, 2002). Because of the limited historical control data available and the lack of a dose-related response in the current study, the relationship between forestomach papillomas and trimethylolpropane triacrylate administration is uncertain.

Another systemic change diagnosed in several dosed male and female TG.AC mice involved one to several organs and had characteristics that variably resembled hematopoietic, inflammatory, and neoplastic processes. The most consistent presentation was an aberrant infiltration and/or proliferation of granulocyte-rich inflammatory cells. The major granulocyte component was the eosinophil, an inflammatory cell type usually associated with immune reactions or parasitic infections. The liver was the organ most frequently affected, although other tissues (lymph nodes, spleen, lung, kidney and epididymis) were also affected. The bone marrow, a site that would be expected to be affected, was not microscopically examined in these studies. Because of the variable morphology and uncertain biological behavior, assigning an appropriate diagnostic term was problematic. In its more severe form, the lesion may have resulted from infiltration and/or proliferation, and was diagnosed as myelodysplasia (Mahler *et al.*, 1998). In milder cases, component cells appeared more infiltrative and the change was diagnosed as cellular infiltration. Separating the milder lesions from inflammation and extramedullary hematopoiesis was difficult.

This lesion complex, even in the severe state, appears to be nonneoplastic. This determination is based primarily on morphologic criteria used to distinguish granulocytic hyperplasia and granulocytic leukemia (Long *et al.*, 1986), including the presence of granulocytes in multiple stages of maturation, as well as cells from other lineages such as megakaryocytes. In addition, recent studies suggest that myelodysplasia is a reversible lesion when the inciting chemical stimulus is withdrawn (C. Trempus, personal communication), further suggesting that it is a nonneoplastic process. Myelodysplasia has been previously reported in only one other Tg.AC mouse study of rotenone (Mahler *et al.*, 1998). It has not been observed in untreated Tg.AC mice, and was not present in the vehicle controls in the current study. It has not been reported in other strains of mice; however, in subsequent studies of rotenone involving the Tg.AC and the parent FVB/N strain, it was identified in both (R. Maronpot, personal communication). In the rotenone study, epidermal hyperplasia and dermal inflammation were found at the site of application, but squamous papillomas were not induced, indicating that epidermal tumors are not required for the induction of myelodysplasia (Eastin *et al.*, 1998). Since *ras* mutations are associated with myeloproliferative disorders (Liu, 1990), the activated v-Ha-*ras* oncogene in the genome of Tg.AC mice may predispose this strain to exuberant hematopoietic proliferation and infiltration, and myelodysplasia may be one manifestation of this genomic defect, triggered by an inflammatory stimulus (e.g., the skin). It is also possible that myelodysplasia represents an atypical hypersensitivity reaction in this strain. Clearly additional studies are needed to further clarify the biology of this lesion.

Hematopoietic cell proliferation occurred in the liver, spleen, and lymph nodes of animals in the 6 and 12 mg/kg groups. This reaction may have been at least partially due to the inflammatory stimulus at the skin site of application. Enhanced expression of hematopoietic cytokines by mouse epidermal tumor cells has also been demonstrated (Bauluz *et al.*, 1994), suggesting that some of this response may have been due to treatment-induced squamous papillomas at the site of application.

The NTP has studied two monofunctional acrylates, ethyl acrylate (NTP, 1986a) and methyl methacrylate (NTP, 1986b) for carcinogenicity. Ethyl acrylate administered by gavage produced squamous cell papillomas in the forestomach of rats and mice at doses that also induced considerable nonneoplastic pathology. Interestingly, ethyl acrylate was negative when dermally administered in the Tg.AC model (Nylander-French and French, 1998) and positive when administered by gavage to a short-term *ras* model, the *ras* H2 mouse (Yamamoto *et al.*, 1998). Methyl methacrylate was not carcinogenic in rats or conventional mice when administered via inhalation for 2 years (NTP, 1986b). Of the eight multifunctional acrylates studied by the

Celanese Corporation, two induced skin tumors at the site of application (Andrews and Clary, 1986). All these studies suggest that carcinogenic activity of acrylates is expressed at the site of application only.

Repeated dermal administration of trimethylolpropane triacrylate in the current study caused sustained epidermal cell proliferation and led to formation of papillomas, a major characteristic of the known neoplasm promoters. The evidence of a limited and weak mutagenic response with trimethylolpropane triacrylate in the *Salmonella* assay (Cameron *et al.*, 1991) might support a nongenotoxic mode of action for this chemical. However, the positive results from other *in vitro* genotoxicity assays employing endpoints that are associated with chromosomal breakage events (Dearfield *et al.,* 1989; Moore *et al.,* 1989; Cameron *et al.*, 1991) also indicate a potential for a genotoxic mode of action. Based on the current evidence, it is difficult to classify trimethylolpropane triacrylate as either a genotoxic or nongenotoxic carcinogen. Additional studies are needed to understand the mechanism of skin neoplasm formation by trimethylolpropane triacrylate.

CONCLUSIONS

Male and female Tg.AC hemizygous mice dosed with trimethylolpropane triacrylate for 6 months had significantly increased incidences and multiplicity of papillomas of the skin at the site of dermal application. Treatment-related squamous cell carcinomas occurred at the site of application in dosed female mice. Increased incidences of forestomach squamous cell papilloma in female mice may have been related to chemical administration.

Increased incidences of minimal to moderate (mostly mild) hyperplasia of the epidermis, hyperkeratosis, and chronic active inflammation also occurred at the site of application. A hematopoietic disorder (myelodysplasia) also occurred in dosed male and female mice.

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APPENDIX A
SUMMARY OF LESIONS
IN MALE Tg.AC HEMIZYGOUS MICE
IN THE 6-MONTH DERMAL STUDY
OF TRIMETHYLOLPROPANE TRIACRYLATE

TABLE A1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Natural deaths	1		3	1	2	4
Survivors						
Terminal sacrifice	14	15	12	14	13	11
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Salivary glands			(1)	(1)		(2)
Carcinoma			1 (100%)	1 (100%)		1 (50%)
Stomach, forestomach	(15)	(15)	(15)	(15)	(15)	(15)
Squamous cell papilloma	3 (20%)	4 (27%)	1 (7%)	2 (13%)	2 (13%)	2 (13%)
Squamous cell papilloma, multiple	2 (13%)	3 (20%)		2 (13%)	1 (7%)	
Tooth	(1)	(3)	(1)	(2)	(1)	(1)
Odontogenic tumor	1 (100%)	3 (100%)	1 (100%)		1 (100%)	1 (100%)
Cardiovascular System						
Heart	(15)	(15)	(15)	(15)	(15)	(15)
Endocrine System						
Pituitary gland	(15)	(15)	(15)	(14)	(15)	(15)
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Lymph node	(13)	(13)	(14)	(15)	(14)	(14)
Lymph node, mandibular	(15)	(14)	(15)	(15)	(14)	(13)
Lymph node, mesenteric	(15)	(14)	(14)	(15)	(15)	(12)
Spleen	(15)	(15)	(15)	(15)	(15)	(15)
Thymus	(15)	(15)	(14)	(15)	(15)	(15)
Integumentary System						
Skin	(15)	(15)	(15)	(15)	(15)	(15)
Squamous cell papilloma						2 (13%)
Skin, site of application, squamous cell papilloma				2 (13%)		
Skin, site of application, squamous cell papilloma, multiple					12 (80%)	13 (87%)
Subcutaneous tissue, fibrosarcoma			1 (7%)			

TABLE A1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(14)
Special Senses System						
None						
Urinary System						
Kidney	(15)	(15)	(15)	(15)	(15)	(15)
Systemic Lesions						
Multiple organs[b]	(15)	(15)	(15)	(15)	(15)	(15)
Leukemia erythrocytic						1 (7%)
Lymphoma malignant				1 (7%)		
Neoplasm Summary						
Total animals with primary neoplasms[c]	6	8	4	6	12	13
Total primary neoplasms	6	10	4	8	16	20
Total animals with benign neoplasms	5	7	1	5	12	13
Total benign neoplasms	5	7	1	6	15	17
Total animals with malignant neoplasms			2	1		2
Total malignant neoplasms			2	2		2
Total animals with uncertain neoplasms-benign or malignant	1	3	1		1	1
Total uncertain neoplasms	1	3	1		1	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: Vehicle Control

Number of Days on Study	1 0 1	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	
Carcass ID Number	1 8 1	1 8 2	1 8 4	1 8 5	1 8 6	1 9 1	1 9 3	1 8 3	1 8 7	1 8 8	1 8 9	1 9 0	1 9 2	1 9 4	1 9 5	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma						X						X		X		3
Squamous cell papilloma, multiple	X				X											2
Tooth								+								1
Odontogenic tumor								X								1
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Preputial gland										+						1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	13
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
None																
Nervous System																
None																
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: Vehicle Control

Number of Days on Study	1 0 1	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	
Carcass ID Number	1 8 1	1 8 2	1 8 4	1 8 5	1 8 6	1 9 1	1 9 3	1 8 3	1 8 7	1 8 8	1 8 9	1 9 0	1 9 2	1 9 4	1 9 5	Total Tissues/ Tumors
Special Senses System																
None																
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 0.75 mg/kg

Number of Days on Study	192	192	192	192	192	192	192	192	192	192	192	192	192	192	192	193	
Carcass ID Number	1966	1977	1988	1999	2000	2001	2003	2004	2005	2006	2007	2008	2009	2010	2012	2010	Total Tissues/ Tumors
Alimentary System																	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma		X			X		X									X	4
Squamous cell papilloma, multiple						X		X	X								3
Tooth							+	+		+							3
Odontogenic tumor							X	X		X							3
Cardiovascular System																	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																	
None																	
Genital System																	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																	
Lymph node	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	13
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	14
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	14
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																	
None																	
Nervous System																	
None																	
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 0.75 mg/kg

Number of Days on Study	192	192	192	192	192	192	192	192	192	192	192	192	192	192	192	193	
Carcass ID Number	196	197	198	199	200	201	203	204	205	206	207	208	209	210	212	210	Total Tissues/ Tumors
Special Senses System																	
None																	
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 1.5 mg/kg

Number of Days on Study	0 7 4	0 9 5	1 5 7	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	
Carcass ID Number	2 1 3	2 1 2	2 1 6	2 1 1	2 1 5	2 1 7	2 1 8	2 2 1	2 2 2	2 2 5	2 1 4	2 1 9	2 2 0	2 2 3	2 2 4	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Salivary glands		+														1
Carcinoma		X														1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma										X						1
Tooth			+													1
Odontogenic tumor			X													1
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	14
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	14
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	14
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Subcutaneous tissue, fibrosarcoma											X					1
Musculoskeletal System																
None																
Nervous System																
None																
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 1.5 mg/kg

Number of Days on Study	0 7 4	0 9 5	1 5 7	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	
Carcass ID Number	2 1 3	2 1 2	2 1 6	2 1 1	2 1 5	2 1 7	2 1 8	2 2 1	2 2 2	2 2 5	2 1 4	2 1 9	2 2 0	2 2 3	2 2 4	Total Tissues/ Tumors
Special Senses System																
None																
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder											+					1
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 3 mg/kg

Number of Days on Study	13292	19222	19222	19222	19222	19222	19222	19222	19222	19223	19233	19233	19233	19233	19243	
Carcass ID Number	23235	22226	22227	22228	22229	23230	23231	23233	23237	23239	23242	23244	23246	23248	24240	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Salivary glands	+															1
Carcinoma	X															1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma			X					X								2
Squamous cell papilloma, multiple		X				X										2
Tooth							+								+	2
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	14
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skin, site of application, squamous cell papilloma		X			X											2
Musculoskeletal System																
None																
Nervous System																
None																
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 3 mg/kg

Number of Days on Study	1 3 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	
Carcass ID Number	2 3 5	2 2 6	2 2 7	2 2 8	2 2 9	2 3 0	2 3 1	2 3 3	2 3 7	2 3 9	2 3 2	2 3 4	2 3 6	2 3 8	2 4 0	Total Tissues/ Tumors
Special Senses System																
None																
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymphoma malignant	X															1

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 6 mg/kg

Number of Days on Study	0 9 2	1 8 0	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	
Carcass ID Number	2 5 3	2 4 3	2 4 1	2 4 2	2 4 5	2 4 7	2 4 8	2 4 9	2 5 2	2 5 5	2 4 4	2 4 6	2 5 0	2 5 1	2 5 4	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Mesentery			+													1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma				X								X				2
Squamous cell papilloma, multiple						X										1
Tooth		+														1
Odontogenic tumor		X														1
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	14
Lymph node, mandibular	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skin, site of application, squamous cell papilloma, multiple		X	X	X		X	X	X	X	X		X	X	X	X	12
Musculoskeletal System																
None																
Nervous System																
None																
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 6 mg/kg

Number of Days on Study	0 9 2	1 8 0	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 2	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	
Carcass ID Number	2 5 3	2 4 3	2 4 1	2 4 2	2 4 5	2 4 7	2 4 8	2 4 9	2 5 2	2 5 5	2 4 4	2 4 6	2 5 0	2 5 1	2 5 4	Total Tissues/ Tumors
Special Senses System																
None																
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 12 mg/kg

Number of Days on Study	161	161	182	191	192	192	192	192	192	192	192	192	192	192	193	
Carcass ID Number	25 8	26 6	26 9	26 7	25 6	25 7	25 9	26 1	26 2	26 4	26 5	26 8	27 0	26 0	26 3	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Mesentery												+				1
Salivary glands					+											1
Carcinoma					X											1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma									X						X	2
Tooth														+		1
Odontogenic tumor														X		1
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Preputial gland					+											1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mandibular	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	13
Lymph node, mesenteric	+	M	+	M	+	+	+	+	M	+	+	+	+	+	+	12
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma												X		X		2
Skin, site of application, squamous cell papilloma, multiple	X	X	X	X	X		X	X	X		X	X	X	X	X	13
Musculoskeletal System																
None																
Nervous System																
None																
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	14

TABLE A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 12 mg/kg

Number of Days on Study	161	161	182	191	192	192	192	192	192	192	192	192	192	192	193	193	
Carcass ID Number	25858	26669	26699	26677	25566	25577	25599	26091	26612	26624	26665	26685	26688	27070	26600	26603	Total Tissues/ Tumors
Special Senses System																	
None																	
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Urinary bladder							+		+					+			3
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Leukemia erythrocytic												X					1

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Skin: Squamous Cell Papilloma						
Overall rate[a]	0/15 (0%)	0/15 (0%)	0/15 (0%)	2/15 (13%)	12/15 (80%)	13/15 (87%)
Adjusted rate[b]	0.0%	0.0%	0.0%	14.0%	85.1%	86.7%
Terminal rate[c]	0/14 (0%)	0/15 (0%)	0/12 (0%)	2/14 (14%)	11/13 (85%)	9/11 (82%)
First incidence (days)	—[e]	—[f]	—	192 (T)	180	161
Poly-3 test[d]	P<0.001	—	—	P=0.234	P<0.001	P<0.001
Stomach (Forestomach): Squamous Cell Papilloma						
Overall rate	5/15 (33%)	7/15 (47%)	1/15 (7%)	4/15 (27%)	3/15 (20%)	2/15 (13%)
Adjusted rate	33.3%	46.7%	7.9%	27.9%	21.5%	14.3%
Terminal rate	4/14 (29%)	7/15 (47%)	1/12 (8%)	4/14 (29%)	3/13 (23%)	2/11 (18%)
First incidence (days)101	101	192 (T)	192 (T)	192 (T)	192 (T)	192 (T)
Poly-3 test	P=0.095N	P=0.358	P=0.121N	P=0.532N	P=0.387N	P=0.224N
Tooth: Odontogenic Tumor						
Overall rate	1/15 (7%)	3/15 (20%)	1/15 (7%)	0/15 (0%)	1/15 (7%)	1/15 (7%)
Adjusted rate	7.1%	20.0%	7.6%	0.0%	7.1%	7.1%
Terminal rate	1/14 (7%)	3/15 (20%)	0/12 (0%)	0/14 (0%)	0/13 (0%)	1/11 (9%)
First incidence (days)	192 (T)	192 (T)	157	—	180	192 (T)
Poly-3 test	P=0.403N	P=0.320	P=0.745	P=0.497N	P=0.760	P=0.759
All Organs: Erythrocytic Leukemia						
Overall rate	0/15 (0%)	0/15 (0%)	0/15 (0%)	0/15 (0%)	0/15 (0%)	1/15 (7%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	0.0%	7.1%
Terminal rate	0/14 (0%)	0/15 (0%)	0/12 (0%)	0/14 (0%)	0/13 (0%)	1/11 (9%)
First incidence (days)	—	—	—	—	—	192 (T)
Poly-3 test	P=0.106	—	—	—	—	P=0.498
All Organs: Malignant Lymphoma						
Overall rate	0/15 (0%)	0/15 (0%)	0/15 (0%)	1/15 (7%)	0/15 (0%)	0/15 (0%)
Adjusted rate	0.0%	0.0%	0.0%	6.7%	0.0%	0.0%
Terminal rate	0/14 (0%)	0/15 (0%)	0/12 (0%)	0/14 (0%)	0/13 (0%)	0/11 (0%)
First incidence (days)	—	—	—	132	—	—
Poly-3 test	P=0.695N	—	—	P=0.512	—	—
All Organs: Benign Neoplasms						
Overall rate	5/15 (33%)	7/15 (47%)	1/15 (7%)	5/15 (33%)	12/15 (80%)	13/15 (87%)
Adjusted rate	33.3%	46.7%	7.9%	34.9%	85.1%	86.7%
Terminal rate	4/14 (29%)	7/15 (47%)	1/12 (8%)	5/14 (36%)	11/13 (85%)	9/11 (82%)
First incidence (days)	101	192 (T)	192 (T)	192 (T)	180	161
Poly-3 test	P<0.001	P=0.358	P=0.121N	P=0.614	P=0.003	P<0.001
All Organs: Malignant Neoplasms						
Overall rate	0/15 (0%)	0/15 (0%)	2/15 (13%)	1/15 (7%)	0/15 (0%)	2/15 (13%)
Adjusted rate	0.0%	0.0%	14.7%	6.7%	0.0%	14.3%
Terminal rate	0/14 (0%)	0/15 (0%)	1/12 (8%)	0/14 (0%)	0/13 (0%)	2/11 (18%)
First incidence (days)	—	—	95	132	—	192 (T)
Poly-3 test	P=0.182	—	P=0.222	P=0.512	—	P=0.229

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
All Organs: Benign or Malignant Neoplasms						
Overall rate	6/15 (40%)	8/15 (53%)	4/15 (27%)	6/15 (40%)	12/15 (80%)	13/15 (87%)
Adjusted rate	40.0%	53.3%	28.5%	40.0%	85.1%	86.7%
Terminal rate	5/14 (36%)	8/15 (53%)	2/12 (17%)	5/14 (36%)	11/13 (85%)	9/11 (82%)
First incidence (days)	101	192 (T)	95	132	180	161
Poly-3 test	P<0.001	P=0.361	P=0.399N	P=0.641	P=0.011	P<0.006

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined microscopically for skin; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Natural deaths	1		3	1	2	4
Survivors						
Terminal sacrifice	14	15	12	14	13	11
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Basophilic focus		1 (7%)				
Hematopoietic cell proliferation	1 (7%)		1 (7%)			6 (40%)
Infarct		1 (7%)				
Infiltration cellular						5 (33%)
Inflammation, acute		1 (7%)				
Inflammation, chronic active	7 (47%)	10 (67%)	8 (53%)	8 (53%)	7 (47%)	8 (53%)
Mineralization		1 (7%)				
Myelodysplasia						1 (7%)
Pigmentation		2 (13%)				
Hepatocyte, necrosis	2 (13%)	2 (13%)	2 (13%)		1 (7%)	1 (7%)
Mesentery					(1)	(1)
Fat, necrosis					1 (100%)	1 (100%)
Salivary glands			(1)	(1)		(2)
Mandibular, infiltration cellular						1 (50%)
Stomach, forestomach	(15)	(15)	(15)	(15)	(15)	(15)
Hyperkeratosis			1 (7%)		1 (7%)	3 (20%)
Inflammation, chronic active					1 (7%)	
Epithelium, hyperplasia					1 (7%)	
Tooth	(1)	(3)	(1)	(2)	(1)	(1)
Inflammation, chronic active				1 (50%)		
Cardiovascular System						
Heart	(15)	(15)	(15)	(15)	(15)	(15)
Atrium, thrombosis			1 (7%)			
Endocrine System						
Adrenal cortex	(15)	(15)	(15)	(15)	(15)	(15)
Hyperplasia						1 (7%)
Hypertrophy	7 (47%)	5 (33%)	9 (60%)	8 (53%)	5 (33%)	5 (33%)
Vacuolization cytoplasmic						1 (7%)
Pituitary gland	(15)	(15)	(15)	(14)	(15)	(15)
Pars distalis, cyst	2 (13%)	1 (7%)	1 (7%)	1 (7%)	1 (7%)	2 (13%)
Thyroid gland	(15)	(15)	(15)	(15)	(15)	(15)
Inflammation, chronic active						1 (7%)
Follicle, cyst			1 (7%)			
General Body System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Genital System						
Epididymis	(15)	(15)	(15)	(15)	(15)	(15)
Atrophy		1 (7%)				
Inflammation, chronic active		2 (13%)				3 (20%)
Myelodysplasia						1 (7%)
Bilateral, hypospermia		1 (7%)				
Unilateral, hypospermia	4 (27%)		3 (20%)	1 (7%)	1 (7%)	2 (13%)
Preputial gland	(1)					(1)
Inflammation, chronic active						1 (100%)
Duct, ectasia	1 (100%)					
Testes	(15)	(15)	(15)	(15)	(15)	(15)
Cyst	2 (13%)		1 (7%)			1 (7%)
Bilateral, germinal epithelium, degeneration		1 (7%)				
Germinal epithelium, degeneration						1 (7%)
Unilateral, germinal epithelium, degeneration	4 (27%)		3 (20%)	1 (7%)	1 (7%)	2 (13%)
Hematopoietic System						
Lymph node	(13)	(13)	(14)	(15)	(14)	(14)
Hyperplasia						4 (29%)
Inflammation, chronic active						3 (21%)
Axillary, infiltration cellular						1 (7%)
Axillary, infiltration cellular, plasma cell						1 (7%)
Inguinal, hyperplasia						1 (7%)
Inguinal, inflammation, chronic active						1 (7%)
Mediastinal, hematopoietic cell proliferation						1 (7%)
Mediastinal, hyperplasia						1 (7%)
Mediastinal, infiltration cellular						3 (21%)
Mediastinal, infiltration cellular, plasma cell						3 (21%)
Mediastinal, inflammation, chronic active						2 (14%)
Lymph node, mandibular	(15)	(14)	(15)	(15)	(14)	(13)
Hematopoietic cell proliferation						2 (15%)
Hyperplasia		1 (7%)				1 (8%)
Hyperplasia, plasma cell			1 (7%)		1 (7%)	
Infiltration cellular						4 (31%)
Infiltration cellular, plasma cell						1 (8%)
Lymph node, mesenteric	(15)	(14)	(14)	(15)	(15)	(12)
Infiltration cellular						1 (8%)
Spleen	(15)	(15)	(15)	(15)	(15)	(15)
Atrophy			1 (7%)		1 (7%)	4 (27%)
Depletion cellular			1 (7%)		1 (7%)	
Hematopoietic cell proliferation	1 (7%)	4 (27%)	1 (7%)	2 (13%)	6 (40%)	8 (53%)
Hyperplasia, lymphoid		1 (7%)	1 (7%)	1 (7%)	1 (7%)	
Thymus	(15)	(15)	(14)	(15)	(15)	(15)
Atrophy	1 (7%)		1 (7%)		2 (13%)	5 (33%)
Thymocyte, necrosis						1 (7%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Integumentary System						
Skin	(15)	(15)	(15)	(15)	(15)	(15)
Epidermis, skin, site of application, hyperplasia				6 (40%)	14 (93%)	15 (100%)
Epidermis, skin, site of application, hyperplasia, focal			1 (7%)		1 (7%)	
Skin, site of application, hyperkeratosis			1 (7%)	15 (100%)	14 (93%)	12 (80%)
Skin, site of application, hyperplasia						1 (7%)
Skin, site of application, inflammation, chronic active			1 (7%)	1 (7%)	9 (60%)	12 (80%)
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(14)
Inflammation, chronic active		2 (13%)				2 (14%)
Thrombosis				1 (7%)		
Interstitium, hyperplasia	1 (7%)					
Interstitium, inflammation, chronic active			1 (7%)			
Mediastinum, infiltration cellular						1 (7%)
Mediastinum, inflammation, chronic active						1 (7%)
Special Senses System						
None						
Urinary System						
Kidney	(15)	(15)	(15)	(15)	(15)	(15)
Infarct					1 (7%)	1 (7%)
Infiltration cellular						1 (7%)
Infiltration cellular, mononuclear cell	1 (7%)	1 (7%)				1 (7%)
Inflammation, chronic active		2 (13%)	1 (7%)		1 (7%)	5 (33%)
Mineralization					1 (7%)	
Nephropathy	3 (20%)	6 (40%)				3 (20%)
Glomerulus, renal tubule, dilatation					1 (7%)	
Renal tubule, cyst	1 (7%)	1 (7%)				1 (7%)
Renal tubule, hyperplasia	1 (7%)		1 (7%)		1 (7%)	1 (7%)
Renal tubule, regeneration	2 (13%)	1 (7%)	5 (33%)	4 (27%)	4 (27%)	

TABLE A5
In-Life Observation of Skin Papilloma at the Site of Application in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 6 mg/kg[a]

Carcass ID Number	24 1	24 2	24 3	24 4	24 5	24 6	24 7	24 8	24 9	25 0	25 1	25 2	25 3	25 4	25 5	Total Tumors	Animals with Tumors
Week																	
14	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1
15	0	0	0	0	0	0	0	1	0	0	0	0	X	0	0	1	1
16	0	0	0	0	0	0	0	1	0	0	0	0		7	0	8	2
17	2	0	0	0	0	1	0	1	0	0	0	0		6	0	10	4
18	2	0	0	0	0	0	0	1	0	0	0	0		6	0	9	3
19	2	0	9	0	2	0	0	1	0	1	0	0		6	0	21	6
20	2	0	11	0	1	0	0	1	0	1	0	4		8	0	28	7
21	3	0	8	0	2	0	0	2	0	1	0	5		14	0	35	7
22	3	0	13	0	2	0	1	2	0	2	0	9		16	0	48	8
23	4	0	12	0	2	0	2	8	6	3	2	7		20	0	≥66	10
24	4	0	13	0	2	0	2	8	5	4	2	9		16	0	65	10
25	4	0	13	0	2	0	3	8	6	6	2	15		20	4	≥83	11
26	4	0	20	0	0	3	3	8	7	7	3	15		20	4	≥94	11
27	4	0	X	0	0	3	6	13	6	8	5	20		20	6	≥91	10
28	3	0		0	0	3	6	12	6	8	4	20		20	8	≥90	10
Necropsy	3	5	17	0	0	2	6	14	7	8	4	20	0	20	9	≥115	12

TABLE A5
In-Life Observation of Skin Papilloma at the Site of Application in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 12 mg/kg

Carcass ID Number	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	Total Tumors	Animals with Tumors
Week																	
9	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	3	1
10	0	0	0	0	2	0	0	1	0	0	3	0	0	4	0	10	4
11	0	0	0	0	3	0	0	1	0	0	2	0	0	3	0	9	4
12	0	0	0	0	3	0	0	1	0	1	7	0	0	4	5	21	6
13	5	0	1	0	8	0	5	1	0	2	7	0	0	5	6	40	9
14	9	0	1	0	8	0	6	3	0	3	6	0	7	4	8	55	10
15	12	0	3	7	11	0	9	3	0	5	11	5	12	12	12	102	12
16	14	0	3	12	10	16	9	5	0	5	12	10	13	11	10	130	13
17	20	0	6	15	13	20	13	7	0	5	13	14	17	15	12	≥170	13
18	20	0	12	16	13	20	15	15	0	6	20	20	20	20	13	≥210	13
19	20	0	10	18	17	20	14	20	0	7	20	20	20	20	16	≥222	13
20	20	0	15	15	20	20	20	20	0	6	20	20	20	20	20	≥236	13
21	20	0	20	20	20	20	20	20	0	4	20	20	20	20	20	≥244	13
22	20	0	15	20	20	20	20	20	0	7	20	20	20	20	20	≥242	13
23	20	0	20	20	20	20	20	20	0	13	20	20	20	20	20	≥253	13
24	20	0	X	20	20	20	20	20	0	20	X	20	20	20	20	≥220	11
25	20	0		20	20	20	20	20	0	20		20	20	20	20	≥220	11
26	20	0		20	20	20	20	20	0	20		20	20	20	20	≥220	11
27	20	0		20	20	20	20	20	0	20		20	20	X	20	≥200	10
28	20	0		20	20	20	20	20	0	20		20	20		20	≥200	10
Necropsy	20	0	20	20	20	20	20	20	0	20	20	20	20	20	20	≥260	13

X=animal died

[a] No papillomas occurred in the vehicle control, 0.75, or 1.5 mg/kg groups. In the 3 mg/kg group, animal 226 had a single papilloma first observed at necropsy; animal 229 had a single papilloma observed at week 28 and at necropsy, and animal 232 had a single papilloma observed only at week 23. The maximum number of papillomas reported in the 6 and 12 mg/kg groups was 20, although some mice in these groups had more than 20 papillomas. Animal 268 in the 12 mg/kg group died between week 28 and scheduled necropsy.

APPENDIX B
SUMMARY OF LESIONS
IN FEMALE Tg.AC HEMIZYGOUS MICE
IN THE 6-MONTH DERMAL STUDY
OF TRIMETHYLOLPROPANE TRIACRYLATE

Table B1
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund			1			
Natural deaths		1	2	1	1	3
Survivors						
Terminal sacrifice	15	14	12	14	14	12
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Salivary glands	(1)					(1)
Carcinoma	1 (100%)					
Stomach, forestomach	(15)	(15)	(15)	(15)	(15)	(15)
Squamous cell papilloma	3 (20%)	4 (27%)	3 (20%)	1 (7%)	4 (27%)	6 (40%)
Squamous cell papilloma, multiple	1 (7%)	1 (7%)	1 (7%)	1 (7%)	1 (7%)	3 (20%)
Tooth	(1)	(4)	(3)	(1)	(3)	(2)
Odontogenic tumor	1 (100%)	4 (100%)	2 (67%)		3 (100%)	1 (50%)
Cardiovascular System						
Heart	(15)	(15)	(15)	(15)	(15)	(15)
Endocrine System						
Pituitary gland	(15)	(15)	(15)	(15)	(15)	(15)
General Body System						
None						
Genital System						
Ovary	(15)	(15)	(15)	(15)	(15)	(15)
Uterus	(15)	(15)	(15)	(15)	(15)	(15)
Vagina		(1)				
Squamous cell papilloma		1 (100%)				
Hematopoietic System						
Lymph node	(11)	(14)	(13)	(10)	(13)	(12)
Lymph node, mandibular	(15)	(15)	(15)	(15)	(15)	(15)
Lymph node, mesenteric	(15)	(14)	(14)	(15)	(15)	(15)
Spleen	(15)	(15)	(15)	(15)	(15)	(15)
Thymus	(15)	(15)	(15)	(15)	(15)	(15)

Table B1
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Integumentary System						
Skin	(15)	(15)	(15)	(15)	(15)	(15)
Squamous cell papilloma		1 (7%)		1 (7%)		
Skin, site of application, squamous cell carcinoma			1 (7%)		1 (7%)	1 (7%)
Skin, site of application, squamous cell papilloma				1 (7%)	6 (40%)	
Skin, site of application, squamous cell papilloma, multiple					5 (33%)	15 (100%)
Vulva, squamous cell papilloma					1 (7%)	
Musculoskeletal System						
Bone		(1)				
Osteosarcoma		1 (100%)				
Nervous System						
None						
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(15)
Alveolar/bronchiolar adenoma	1 (7%)			1 (7%)		
Special Senses System						
None						
Urinary System						
Kidney	(15)	(15)	(15)	(15)	(15)	(15)
Systemic Lesions						
Multiple organs[b]	(15)	(15)	(15)	(15)	(15)	(15)
Leukemia erythrocytic		1 (7%)		1 (7%)		2 (13%)
Lymphoma malignant						1 (7%)
Neoplasm Summary						
Total animals with primary neoplasms[c]	6	9	6	5	12	15
Total primary neoplasms	7	13	7	6	21	29
Total animals with benign neoplasms	5	5	4	4	11	15
Total benign neoplasms	5	7	4	5	17	24
Total animals with malignant neoplasms	1	2	1	1	1	3
Total malignant neoplasms	1	2	1	1	1	4
Total animals with uncertain neoplasms-benign or malignant	1	4	2		3	1
Total uncertain neoplasms	1	4	2		3	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: Vehicle Control

Number of Days on Study	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	
Carcass ID Number	2 7 2	2 7 3	2 7 8	2 8 1	2 8 2	2 8 3	2 8 5	2 7 1	2 7 4	2 7 5	2 7 6	2 7 7	2 7 9	2 8 0	2 8 4	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Salivary glands												+				1
Carcinoma												X				1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma				X	X			X								3
Squamous cell papilloma, multiple						X										1
Tooth												+				1
Odontogenic tumor												X				1
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	+	+	M	+	+	+	+	+	M	M	M	+	+	+	+	11
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Musculoskeletal System																
None																
Nervous System																
None																
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Alveolar/bronchiolar adenoma															X	1

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: Vehicle Control

Number of Days on Study	193	193	193	193	193	193	193	194	194	194	194	194	194	194	194	194	
Carcass ID Number	27272	27273	27278	27281	27282	27283	27285	27171	27174	27175	27176	27177	27179	27180	27184		Total Tissues/ Tumors
Special Senses System																	
None																	
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 0.75 mg/kg

Number of Days on Study	183	193	193	193	194	194	194	194	194	194	194	194	194	194	194	194	
Carcass ID Number	28 86	29 02	29 06	29 09	28 87	28 88	28 89	29 00	29 01	29 03	29 04	29 05	29 07	29 08	30 00		Total Tissues/ Tumors
Alimentary System																	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Squamous cell papilloma					X				X	X		X					4
Squamous cell papilloma, multiple							X										1
Tooth			+			+				+		+					4
Odontogenic tumor			X			X				X		X					4
Cardiovascular System																	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
General Body System																	
None																	
Genital System																	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Vagina												+					1
Squamous cell papilloma												X					1
Hematopoietic System																	
Lymph node	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+		14
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Integumentary System																	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Squamous cell papilloma					X												1
Musculoskeletal System																	
Bone	+																1
Osteosarcoma	X																1
Nervous System																	
None																	
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 0.75 mg/kg

Number of Days on Study	183	193	193	193	194	194	194	194	194	194	194	194	194	194	194	194	
Carcass ID Number	28626	29292	29966	29969	28877	28889	28909	29090	29191	29293	29294	29295	29297	29298	29300		Total Tissues/ Tumors
Special Senses System																	
None																	
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Leukemia erythrocytic								X									1

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 1.5 mg/kg

Number of Days on Study	0 6 3	0 6 3	1 2 2	1 9 3	1 9 3	1 9 3	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	
Carcass ID Number	3 0 0 4	3 0 0 7	3 1 0 0	3 0 2 0	3 1 2 2	3 1 1 5	3 0 1 1	3 0 0 3	3 0 0 5	3 0 0 6	3 0 0 8	3 0 0 9	3 1 1 1	3 1 1 3	3 1 1 4	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma				X						X					X	3
Squamous cell papilloma, multiple													X			1
Tooth	+				+										+	3
Odontogenic tumor					X										X	2
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	13
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	14
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skin, site of application, squamous cell carcinoma											X					1
Musculoskeletal System																
None																
Nervous System																
None																
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 1.5 mg/kg

Number of Days on Study	0 6 3	0 6 3	1 2 2	1 9 3	1 9 3	1 9 3	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	
Carcass ID Number	3 0 4	3 0 7	3 1 0	3 0 2	3 1 2	3 1 5	3 0 1	3 0 3	3 0 5	3 0 6	3 0 8	3 0 9	3 1 1	3 1 3	3 1 4	Total Tissues/ Tumors
Special Senses System																
None																
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 3 mg/kg

Number of Days on Study	1 2 4	1 9 3	1 9 3	1 9 3	1 9 3	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	
Carcass ID Number	3 2 2	3 1 6	3 1 8	3 2 0	3 2 1	3 1 7	3 1 9	3 2 3	3 2 4	3 2 5	3 2 6	3 2 7	3 2 8	3 2 9	3 3 0	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma														X		1
Squamous cell papilloma, multiple			X													1
Tooth				+												1
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	+	M	M	+	+	+	+	M	+	+	M	+	+	+	M	10
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma			X													1
Skin, site of application, squamous cell papilloma						X										1
Musculoskeletal System																
None																
Nervous System																
None																
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Alveolar/bronchiolar adenoma									X							1

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 3 mg/kg

Number of Days on Study	1 2 4	1 9 3	1 9 3	1 9 3	1 9 3	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	
Carcass ID Number	3 2 2	3 1 6	3 1 8	3 2 0	3 2 1	3 1 7	3 1 9	3 2 3	3 2 4	3 2 5	3 2 6	3 2 7	3 2 8	3 2 9	3 3 0	Total Tissues/ Tumors
Special Senses System																
None																
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Leukemia erythrocytic												X				1

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 6 mg/kg

Number of Days on Study	1 6 2	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	
Carcass ID Number	3 4 5	3 3 1	3 3 6	3 3 7	3 3 8	3 3 9	3 4 0	3 3 2	3 3 3	3 3 4	3 3 5	3 4 1	3 4 2	3 4 3	3 4 4		Total Tissues/ Tumors
Alimentary System																	
Esophagus			+			+			+								3
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Squamous cell papilloma		X								X		X	X				4
Squamous cell papilloma, multiple						X											1
Tooth	+	+						+									3
Odontogenic tumor	X	X						X									3
Cardiovascular System																	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Endocrine System																	
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
General Body System																	
None																	
Genital System																	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Hematopoietic System																	
Lymph node	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+		13
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Integumentary System																	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Skin, site of application, squamous cell carcinoma										X							1
Skin, site of application, squamous cell papilloma		X		X			X		X	X			X				6
Skin, site of application, squamous cell papilloma, multiple	X		X			X					X	X					5
Vulva, squamous cell papilloma												X					1
Musculoskeletal System																	
None																	
Nervous System																	
None																	
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 6 mg/kg

Number of Days on Study	1 6 2	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 3	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	1 9 4	
Carcass ID Number	3 4 5	3 3 1	3 3 6	3 3 7	3 3 8	3 3 9	3 4 0	3 3 2	3 3 3	3 3 4	3 3 5	3 4 1	3 4 2	3 4 3	3 4 4	Total Tissues/ Tumors	
Special Senses System																	
None																	
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15	
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15	

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 12 mg/kg

Number of Days on Study	118	176	189	193	193	193	193	194	194	194	194	194	194	194	194	
Carcass ID Number	35353	35558	35487	44878	44989	45191	46016	45020	45202	45424	45646	45779	45949	46060		Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Salivary glands						+										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma					X		X			X	X		X	X		6
Squamous cell papilloma, multiple						X			X			X				3
Tooth			+					+								2
Odontogenic tumor								X								1
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	+	+	+	M	M	+	+	+	+	+	+	M	+	+	+	12
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Skin, site of application, squamous cell carcinoma	X															1
Skin, site of application, squamous cell papilloma, multiple	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	15
Musculoskeletal System																
None																
Nervous System																
None																
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

TABLE B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 12 mg/kg

Number of Days on Study	118	176	189	193	193	193	193	194	194	194	194	194	194	194	194	
Carcass ID Number	35303	35505	35508	34707	34808	34909	35101	34606	35400	35502	35504	35506	35507	35509	36000	Total Tissues/ Tumors
Special Senses System																
Eye									+							1
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Leukemia erythrocytic	X	X														2
Lymphoma malignant										X						1

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Skin: Squamous Cell Papilloma						
Overall rate[a]	0/15 (0%)	0/15 (0%)	0/15 (0%)	1/15 (7%)	11/15 (73%)	15/15 (100%)
Adjusted rate[b]	0.0%	0.0%	0.0%	7.0%	73.3%	100.0%
Terminal rate[c]	0/15 (0%)	0/14 (0%)	0/12 (0%)	1/14 (7%)	10/14 (71%)	12/12 (100%)
First incidence (days)	—[e]	—[f]	—	193 (T)	162	118
Poly-3 test[d]	P<0.001	—	—	P=0.490	P<0.001	P<0.001
Skin: Squamous Cell Papilloma or Squamous Cell Carcinoma						
Overall rate	0/15 (0%)	1/15 (7%)	1/15 (7%)	2/15 (13%)	11/15 (73%)	15/15 (100%)
Adjusted rate	0.0%	6.7%	8.1%	14.0%	73.3%	100.0%
Terminal rate	0/15 (0%)	1/14 (7%)	1/12 (8%)	2/14 (14%)	10/14 (71%)	12/12 (100%)
First incidence (days)	—	193 (T)	193 (T)	193 (T)	162	118
Poly-3 test	P<0.001	P=0.498	P=0.461	P=0.220	P<0.001	P<0.001
Stomach (Forestomach): Squamous Cell Papilloma						
Overall rate	4/15 (27%)	5/15 (33%)	4/15 (27%)	2/15 (13%)	5/15 (33%)	9/15 (60%)
Adjusted rate	26.7%	33.7%	32.5%	14.0%	34.3%	64.6%
Terminal rate	4/15 (27%)	5/14 (36%)	4/12 (33%)	2/14 (14%)	5/14 (36%)	9/12 (75%)
First incidence (days)	193 (T)	193 (T)	193 (T)	193 (T)	193 (T)	193 (T)
Poly-3 test	P=0.014	P=0.493	P=0.535	P=0.352N	P=0.481	P=0.040
Tooth: Odontogenic Tumor						
Overall rate	1/15 (7%)	4/15 (27%)	2/15 (13%)	0/15 (0%)	3/15 (20%)	1/15 (7%)
Adjusted rate	6.7%	26.9%	16.2%	0.0%	20.0%	7.2%
Terminal rate	1/15 (7%)	4/14 (29%)	2/12 (17%)	0/14 (0%)	2/14 (14%)	1/12 (8%)
First incidence (days)	193 (T)	193 (T)	193 (T)	—	162	193 (T)
Poly-3 test	P=0.397N	P=0.160	P=0.431	P=0.510N	P=0.298	P=0.744
All Organs: Erythrocytic Leukemia						
Overall rate	0/15 (0%)	1/15 (7%)	0/15 (0%)	1/15 (7%)	0/15 (0%)	2/15 (13%)
Adjusted rate	0.0%	6.7%	0.0%	7.0%	0.0%	13.4%
Terminal rate	0/15 (0%)	1/14 (7%)	0/12 (0%)	1/14 (7%)	0/14 (0%)	0/12 (0%)
First incidence (days)	—	193 (T)	—	193 (T)	—	118
Poly-3 test	P=0.137	P=0.498	—	P=0.490	—	P=0.231
All Organs: Malignant Lymphoma						
Overall rate	0/15 (0%)	0/15 (0%)	0/15 (0%)	0/15 (0%)	0/15 (0%)	3/15 (20%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	0.0%	20.1%
Terminal rate	0/15 (0%)	0/14 (0%)	0/12 (0%)	0/14 (0%)	0/14 (0%)	1/12 (8%)
First incidence (days)	—	—	—	—	—	118
Poly-3 test	P<0.001	—	—	—	—	P=0.105
All Organs: Benign Neoplasms						
Overall rate	5/15 (33%)	5/15 (33%)	4/15 (27%)	4/15 (27%)	11/15 (73%)	15/15 (100%)
Adjusted rate	33.3%	33.7%	32.5%	28.0%	73.3%	100.0%
Terminal rate	5/15 (33%)	5/14 (36%)	4/12 (33%)	4/14 (29%)	10/14 (71%)	12/12 (100%)
First incidence (days)	193 (T)	193 (T)	193 (T)	193 (T)	162	118
Poly-3 test	P<0.001	P=0.639	P=0.635N	P=0.535N	P=0.027	P<0.001

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
All Organs: Malignant Neoplasms						
Overall rate	1/15 (7%)	2/15 (13%)	1/15 (7%)	1/15 (7%)	1/15 (7%)	3/15 (20%)
Adjusted rate	6.7%	13.3%	8.1%	7.0%	6.9%	20.1%
Terminal rate	1/15 (7%)	1/14 (7%)	1/12 (8%)	1/14 (7%)	1/14 (7%)	1/12 (8%)
First incidence (days)	193 (T)	183	193 (T)	193 (T)	193 (T)	118
Poly-3 test	P=0.213	P=0.500	P=0.715	P=0.749	P=0.754	P=0.297
All Organs: Benign or Malignant Neoplasms						
Overall rate	6/15 (40%)	9/15 (60%)	6/15 (40%)	5/15 (33%)	12/15 (80%)	15/15 (100%)
Adjusted rate	40.0%	60.0%	48.7%	35.1%	80.0%	100.0%
Terminal rate	6/15 (40%)	8/14 (57%)	6/12 (50%)	5/14 (36%)	11/14 (79%)	12/12 (100%)
First incidence (days)	193 (T)	183	193 (T)	193 (T)	162	118
Poly-3 test	P<0.001	P=0.236	P=0.474	P=0.541N	P=0.024	P<0.001

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined microscopically for skin; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund			1			
Natural deaths		1	2	1	1	3
Survivors						
Terminal sacrifice	15	14	12	14	14	12
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation			1 (7%)			6 (40%)
Infiltration cellular			1 (7%)			1 (7%)
Inflammation, chronic active	15 (100%)	14 (93%)	13 (87%)	13 (87%)	14 (93%)	8 (53%)
Myelodysplasia						2 (13%)
Pigmentation	12 (80%)	15 (100%)	12 (80%)	13 (87%)	14 (93%)	4 (27%)
Hepatocyte, necrosis	4 (27%)	2 (13%)	1 (7%)	2 (13%)	1 (7%)	1 (7%)
Salivary glands	(1)					(1)
Parotid gland, infiltration cellular						1 (100%)
Tooth	(1)	(4)	(3)	(1)	(3)	(2)
Fibrosis						1 (50%)
Inflammation, chronic active				1 (100%)		
Malformation			1 (33%)			
Cardiovascular System						
Heart	(15)	(15)	(15)	(15)	(15)	(15)
Inflammation, chronic active						1 (7%)
Atrium, thrombosis						1 (7%)
Endocrine System						
Pituitary gland	(15)	(15)	(15)	(15)	(15)	(15)
Pars distalis, angiectasis				1 (7%)		
Pars distalis, cyst	1 (7%)		2 (13%)	1 (7%)	2 (13%)	2 (13%)
Thyroid gland	(15)	(15)	(15)	(15)	(15)	(15)
Infiltration cellular						1 (7%)
Inflammation, chronic active						2 (13%)
General Body System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Genital System						
Ovary	(15)	(15)	(15)	(15)	(15)	(15)
Cyst	1 (7%)			1 (7%)		1 (7%)
Inflammation, chronic active				1 (7%)	1 (7%)	
Pigmentation	1 (7%)	1 (7%)				
Uterus	(15)	(15)	(15)	(15)	(15)	(15)
Hydrometra		1 (7%)		1 (7%)	1 (7%)	1 (7%)
Inflammation, chronic active	1 (7%)					
Cervix, inflammation, chronic active		1 (7%)				
Endometrium, hyperplasia, cystic	10 (67%)	11 (73%)	7 (47%)	8 (53%)	8 (53%)	9 (60%)
Hematopoietic System						
Lymph node	(11)	(14)	(13)	(10)	(13)	(12)
Hematopoietic cell proliferation						2 (17%)
Hyperplasia						2 (17%)
Inguinal, hematopoietic cell proliferation						1 (8%)
Inguinal, hyperplasia						1 (8%)
Inguinal, infiltration cellular, plasma cell						2 (17%)
Inguinal, myelodysplasia						2 (17%)
Mediastinal, hematopoietic cell proliferation						5 (42%)
Mediastinal, infiltration cellular			1 (8%)			2 (17%)
Mediastinal, infiltration cellular, plasma cell						2 (17%)
Lymph node, mandibular	(15)	(15)	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation			1 (7%)			6 (40%)
Hyperplasia	1 (7%)	3 (20%)	2 (13%)		2 (13%)	3 (20%)
Hyperplasia, plasma cell			1 (7%)		1 (7%)	
Infiltration cellular			1 (7%)			1 (7%)
Infiltration cellular, plasma cell			1 (7%)			2 (13%)
Inflammation, chronic active			1 (7%)			
Myelodysplasia						1 (7%)
Lymph node, mesenteric	(15)	(14)	(14)	(15)	(15)	(15)
Hematopoietic cell proliferation						4 (27%)
Infiltration cellular						2 (13%)
Spleen	(15)	(15)	(15)	(15)	(15)	(15)
Atrophy		1 (7%)	3 (20%)	1 (7%)	2 (13%)	1 (7%)
Hematopoietic cell proliferation	7 (47%)	13 (87%)	9 (60%)	6 (40%)	8 (53%)	10 (67%)
Hyperplasia, lymphoid	2 (13%)	2 (13%)	2 (13%)			
Infiltration cellular						1 (7%)
Myelodysplasia						2 (13%)
Thymus	(15)	(15)	(15)	(15)	(15)	(15)
Atrophy		1 (7%)	3 (20%)	2 (13%)	2 (13%)	3 (20%)
Thymocyte, necrosis			2 (13%)	1 (7%)		1 (7%)
Integumentary System						
Skin	(15)	(15)	(15)	(15)	(15)	(15)
Epidermis, site of application, hyperplasia			1 (7%)	4 (27%)	15 (100%)	15 (100%)
Epidermis, site of application, hyperplasia, focal				1 (7%)	1 (7%)	8 (53%)
Site of application, hyperkeratosis			1 (7%)	7 (47%)	14 (93%)	13 (87%)
Site of application, hyperplasia					1 (7%)	
Site of application, inflammation, chronic active				3 (20%)	14 (93%)	12 (80%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(15)
Inflammation, chronic active			1 (7%)			
Mediastinum, infiltration cellular						1 (7%)
Special Senses System						
None						
Urinary System						
Kidney	(15)	(15)	(15)	(15)	(15)	(15)
Inflammation, chronic active			2 (13%)			3 (20%)
Mineralization						1 (7%)
Nephropathy	4 (27%)					1 (7%)
Renal tubule, cyst						1 (7%)
Renal tubule, regeneration		1 (7%)			2 (13%)	1 (7%)

TABLE B5
In-Life Observation of Skin Papilloma at the Site of Application in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 6 mg/kg[a]

Carcass ID Number	33331	33332	33333	33334	33335	33336	33337	33338	33339	33340	33341	33342	33343	33344	33345	Total Tumors	Animals with Tumors
Week																	
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1
14	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1
15	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	2	2
16	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	3	2
17	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	3	2
18	0	0	0	0	3	0	0	0	0	2	0	0	0	0	0	5	2
19	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	4	2
20	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	4	2
21	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	4	2
22	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	4	2
23	0	0	3	0	5	0	0	0	0	1	0	0	0	0	0	9	3
24	0	0	2	0	7	3	0	0	0	1	3	0	0	0	0	16	5
25	0	0	2	0	7	4	0	0	3	1	3	1	0	0	X	21	7
26	0	1	2	0	7	4	0	0	3	1	3	1	0	0		22	8
27	0	0	2	0	12	4	0	0	2	1	3	1	0	0		25	7
28	0	0	2	3	10	4	3	0	3	2	4	2	0	0		33	9
Necropsy	1	0	3	2	12	5	3	0	4	1	4	1	0	0	0	36	10

TABLE B5
In-Life Observation of Skin Papilloma at the Site of Application in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate: 12 mg/kg

Carcass ID Number	34601	34704	34808	34909	35005	35101	35202	35303	35405	35515	35616	35717	35818	35919	36020	Total Tumors	Animals with Tumors
Week																	
10	0	0	1	0	3	0	0	0	0	0	0	0	1	0	0	5	3
11	0	0	1	0	5	0	0	0	0	0	0	0	1	0	0	7	3
12	0	0	3	2	7	0	0	0	0	0	0	0	1	0	0	13	4
13	7	0	3	3	8	0	0	3	0	0	0	0	2	0	0	26	6
14	9	5	4	3	11	0	3	7	0	0	8	7	1	0	0	58	10
15	10	7	10	6	13	0	7	9	0	0	10	7	9	0	15	103	11
16	9	6	10	8	14	7	7	11	7	14	11	10	5	0	17	136	14
17	15	7	15	8	20	10	10	17	7	17	20	16	13	8	20	≥203	15
18	20	7	20	20	20	10	20	X	7	15	20	20	20	10	20	≥229	14
19	20	7	20	20	20	10	20		15	20	20	20	20	15	20	≥247	14
20	20	8	20	20	20	13	20		15	20	20	20	20	15	15	≥246	14
21	20	9	20	20	20	10	20		12	20	20	20	20	20	20	≥251	14
22	20	10	20	20	20	12	20		20	20	20	20	20	20	20	≥262	14
23	20	13	20	20	20	16	20		20	20	20	20	20	20	20	≥269	14
24	20	11	20	20	20	20	20		20	20	20	20	20	20	20	≥271	14
25	20	9	20	20	20	20	20		20	20	20	20	20	20	20	≥269	14
26	20	20	20	20	20	20	20		20	X	20	20	20	20	20	≥260	13
27	20	20	20	20	20	20	20		20		20	20	20	20	20	≥260	13
28	20	20	20	20	20	20	20		20		20	20	X	20	20	≥240	12
Necropsy	20	20	20	20	20	20	20	15	20	20	20	20	20	20	20	≥295	15

X=animal died

[a] No papillomas occurred in the vehicle control or 0.75 mg/kg groups. In the 1.5 mg/kg group, animal 308 had a single papilloma observed from week 19 through necropsy. In the 3 mg/kg group, animals 317 and 318 each had a single papilloma first observed at necropsy. The maximum number of papillomas reported in the 12 mg/kg group was 20, although some mice in this group had more than 20 papillomas.

APPENDIX C
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3- and 6-month studies, peripheral blood samples were obtained from male and female B6C3F$_1$ (3-month study) or Tg.AC hemizygous (6-month study) mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were sent to SITEK Research Laboratories, Inc. (Rockville, MD), where they were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of 10 (3-month study) or up to 15 (6-month study) animals per dose group. In addition, the percentage of normochromatic erythrocytes in 1,000 total erythrocytes per animal was determined to provide a measure of chemical-induced bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3- and 6-month studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocol. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

No increase in the frequency of micronucleated NCEs was observed in peripheral blood samples from male or female mice administered dermal applications of 0.75 to 12 mg trimethylolpropane triacrylate/kg body weight for 3 (Table C1) or 6 (Table C2) months. In the 3-month study, ratios of micronucleated polychromatic erythrocytes to NCEs in peripheral blood were unaltered by chemical treatment, indicating an absence of induced bone marrow toxicity. However, in the 6-month study, decreases in the percentages of circulating NCEs among total erythrocytes were noted in 12 mg/kg male and female mice, indicating a stimulation of erythropoiesis and the presence of increased numbers of immature erythrocytes in circulating blood.

TABLE C1
Frequency of Micronuclei in Peripheral Blood Normochromatic Erythrocytes of B6C3F$_1$ Mice Following Dermal Application of Trimethylolpropane Triacrylate for 3 Months[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	NCEs[b] (%)
Male					
Acetone[d]		10	1.25 ± 0.27		98.0 ± 0.1
Trimethylolpropane triacrylate	0.75	10	1.55 ± 0.17	0.2112	97.4 ± 0.2
	1.5	10	0.95 ± 0.19	0.8173	97.8 ± 0.2
	3	10	1.20 ± 0.31	0.5568	97.5 ± 0.2
	6	10	1.10 ± 0.19	0.6693	98.0 ± 0.2
	12	10	0.60 ± 0.10	0.9837	97.8 ± 0.1
			P=0.993[e]		
Female					
Acetone		10	0.60 ± 0.15		97.9 ± 0.2
Trimethylolpropane triacrylate	0.75	10	0.50 ± 0.17	0.6651	98.0 ± 0.2
	1.5	10	0.85 ± 0.18	0.1765	98.3 ± 0.1
	3	10	0.70 ± 0.21	0.3474	98.1 ± 0.1
	6	10	0.65 ± 0.22	0.4207	97.8 ± 0.2
	12	10	0.80 ± 0.17	0.2248	97.9 ± 0.2
			P=0.232		

[a] Study was performed at SITEK Research Laboratories, Inc. The detailed protocol is presented by MacGregor *et al*. (1990).
[b] NCE=normochromatic erythrocyte
[c] Mean ± standard error
[d] Pairwise comparison with the controls, significant at P≤0.005 (ILS, 1990)
[e] Vehicle control
Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE C2
Frequency of Micronuclei in Peripheral Blood Normochromatic Erythrocytes of Tg.AC Hemizygous Mice Following Dermal Application of Trimethylolpropane Triacrylate for 6 Months[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	NCEs[b] (%)
Male					
Acetone[d]		14	2.82 ± 0.28		97.4 ± 0.1
Trimethylolpropane triacrylate	0.75	15	3.23 ± 0.55	0.1837	97.3 ± 0.2
	1.5	12	3.17 ± 0.35	0.2358	97.2 ± 0.1
	3	14	2.11 ± 0.17	0.9559	97.5 ± 0.1
	6	13	1.88 ± 0.30	0.9874	97.2 ± 0.2
	12	11	2.36 ± 0.24	0.8399	93.7 ± 1.2
			P=0.990[e]		
Female					
Acetone		15	1.00 ± 0.18		97.6 ± 0.1
Trimethylolpropane triacrylate	0.75	14	1.32 ± 0.18	0.1274	97.4 ± 0.1
	1.5	12	1.25 ± 0.22	0.1931	97.8 ± 0.1
	3	14	1.21 ± 0.21	0.2187	97.5 ± 0.3
	6	14	1.14 ± 0.16	0.2994	97.7 ± 0.1
	12	12	1.67 ± 0.28	0.0162	90.7 ± 2.2
			P=0.041		

[a] Study was performed at SITEK Research Laboratories, Inc. The detailed protocol is presented by MacGregor *et al*. (1990).
[b] NCE=normochromatic erythrocyte
[c] Mean ± standard error
[d] Pairwise comparison with the controls, significant at P≤0.005 (ILS, 1990)
[e] Vehicle control
Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX D
SUMMARY OF LESIONS IN RATS AND B6C3F$_1$ MICE IN THE 3-MONTH DERMAL STUDIES OF TRIMETHYLOLPROPANE TRIACRYLATE

TABLE D1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine small, ileum	(10)					(10)
Hyperplasia, lymphoid						1 (10%)
Liver	(10)	(2)	(1)	(1)	(3)	(10)
Hepatodiaphragmatic nodule		2 (100%)	1 (100%)	1 (100%)	3 (100%)	1 (10%)
Inflammation, chronic active	1 (10%)					
Vacuolization cytoplasmic	2 (20%)					2 (20%)
Pancreas	(10)					(10)
Atrophy	1 (10%)					
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	5 (50%)					4 (40%)
Endocrine System						
Pituitary gland	(10)					(10)
Hyperplasia, focal	1 (10%)					
Thyroid gland	(10)					(10)
Ultimobranchial cyst	1 (10%)					
General Body System						
None						
Genital System						
Preputial gland	(10)					(10)
Inflammation, chronic active						1 (10%)
Prostate	(10)					(10)
Inflammation, chronic active						1 (10%)
Hematopoietic System						
Lymph node, mandibular	(10)					(10)
Ectasia, lymphoid						1 (10%)
Lymph node, mesenteric	(10)					(10)
Hyperplasia, lymphoid						1 (10%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Dermis, inflammation, chronic active	2 (20%)	1 (10%)		1 (10%)		
Dermis, skin, site of application, fibrosis						1 (10%)
Dermis, skin, site of application, inflammation, chronic active		1 (10%)	3 (30%)	6 (60%)	10 (100%)	10 (100%)
Epidermis, skin, site of application, degeneration			4 (40%)	7 (70%)	9 (90%)	8 (80%)
Epidermis, skin, site of application, hyperplasia		4 (40%)	7 (70%)	10 (100%)	10 (100%)	10 (100%)
Epidermis, skin, site of application, inflammation, suppurative						4 (40%)
Epidermis, skin, site of application, necrosis				1 (10%)		1 (10%)
Sebaceous gland, skin, site of application, hyperplasia			5 (50%)	10 (100%)	10 (100%)	10 (100%)
Skin, site of application, degeneration				1 (10%)		
Skin, site of application, hyperkeratosis			5 (50%)	10 (100%)	10 (100%)	10 (100%)
Musculoskeletal System						
Skeletal muscle						(1)
Abdominal, inflammation, chronic						1 (100%)
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)			(1)	(10)
Hemorrhage	1 (10%)	1 (100%)			1 (100%)	
Inflammation	1 (10%)					
Inflammation, chronic active		1 (100%)				2 (20%)
Nose	(10)					(10)
Respiratory epithelium, developmental malformation						1 (10%)
Trachea	(10)	(1)				(10)
Hemorrhage		1 (100%)				
Special Senses System						
Eye				(1)		
Atrophy				1 (100%)		
Urinary System						
Kidney	(10)					(10)
Nephropathy						3 (30%)

TABLE D2
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(2)	(1)	(3)	(1)	(10)
Hepatodiaphragmatic nodule	1 (10%)	2 (100%)	1 (100%)	3 (100%)	1 (100%)	4 (40%)
Inflammation						1 (10%)
Inflammation, chronic active	9 (90%)					9 (90%)
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	1 (10%)					
Endocrine System						
Thyroid gland	(10)					(10)
Ultimobranchial cyst	1 (10%)					
General Body System						
None						
Genital System						
Ovary	(10)	(1)	(1)	(1)		(10)
Inflammation, chronic	1 (10%)	1 (100%)	1 (100%)	1 (100%)		
Uterus	(10)		(1)	(1)		(10)
Deciduoma NOS				1 (100%)		
Hydrometra	2 (20%)		1 (100%)			4 (40%)
Hematopoietic System						
Spleen	(10)					(10)
Pigmentation	2 (20%)					4 (40%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D2
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Dermis, skin, site of application, granuloma					1 (10%)	
Dermis, skin, site of application, inflammation, chronic active	1 (10%)	2 (20%)	1 (10%)	9 (90%)	8 (80%)	10 (100%)
Epidermis, skin, site of application, degeneration				4 (40%)	7 (70%)	10 100%)
Epidermis, skin, site of application, hyperplasia				7 (70%)	10 (100%)	10 (100%)
Epidermis, skin, site of application, inflammation, suppurative						6 (60%)
Epidermis, skin, site of application, necrosis						5 (50%)
Sebaceous gland, skin, site of application, hyperplasia		1 (10%)	6 (60%)	9 (90%)	10 (100%)	10 (100%)
Skin, site of application, hyperkeratosis			3 (30%)	9 (90%)	10 (100%)	10 (100%)
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Inflammation, chronic active	4 (40%)					2 (20%)
Nose	(10)					(10)
Respiratory epithelium, metaplasia, squamous						1 (10%)
Special Senses System						
Eye					(1)	
Atrophy					1 (100%)	
Urinary System						
Kidney	(10					(10)
Nephroblastoma						1 (10%)
Nephropathy	1 (10%)					1 (10%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)					(10)
Inflammation, suppurative						1 (10%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
None						
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Dermis, skin, site of application, fibrosis						7 (70%)
Dermis, skin, site of application, inflammation, chronic active	1 (10%)		2 (20%)	10 (100%)	9 (90%)	10 (100%)
Epidermis, skin, site of application, degeneration				4 (40%)	8 (80%)	9 (90%)
Epidermis, skin, site of application, hyperplasia			3 (30%)	10 (100%)	10 (100%)	10 (100%)
Epidermis, skin, site of application, inflammation, suppurative			1 (10%)		1 (10%)	8 (80%)
Epidermis, skin, site of application, necrosis			1 (10%)	1 (10%)	2 (20%)	7 (70%)
Sebaceous gland, skin, site of application, hyperplasia				9 (90%)	10 (100%)	10 (100%)
Skin, site of application, hyperkeratosis			3 (30%)	8 (80%)	8 (80%)	10 (100%)
Musculoskeletal System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
None						

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)					(10)
Inflammation, chronic active	2 (20%)					1 (10%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(10)					(10)
Vacuolization cytoplasmic	8 (80%)					9 (90%)
Subcapsular, hyperplasia	7 (70%)					9 (90%)
General Body System						
None						
Genital System						
Ovary	(10)		(1)			(10)
Inflammation, chronic active			1 (100%)			
Hematopoietic System						
None						
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Dermis, inflammation, chronic active				1 (10%)	2 (20%)	
Dermis, skin, site of application, fibrosis					1 (10%)	7 (70%)
Dermis, skin, site of application, inflammation, chronic active			7 (70%)	10 (100%)	10 (100%)	10 (100%)
Epidermis, skin, site of application, degeneration					5 (50%)	9 (90%)
Epidermis, skin, site of application, hyperplasia			3 (30%)	10 (100%)	9 (90%)	10 (100%)
Epidermis, skin, site of application, inflammation, suppurative			1 (10%)	1 (10%)	2 (20%)	5 (50%)
Epidermis, skin, site of application, necrosis				1 (10%)	2 (20%)	8 (80%)
Sebaceous gland, skin, site of application, hyperplasia			1 (10%)	10 (100%)	10 (100%)	10 (100%)
Skin, site of application, hyperkeratosis			3 (30%)	10 (100%)	9 (90%)	8 (80%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
None						

APPENDIX E
CLINICAL PATHOLOGY RESULTS

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	9	10	10
Day 23	9	10	10	9	10	10
Week 14	9	10	10	10	10	9
Hematocrit (%)						
Day 4	39.1 ± 0.4	38.3 ± 0.5	37.1 ± 0.4*	37.7 ± 0.5	38.6 ± 0.41	39.3 ± 0.5
Day 23	43.5 ± 0.5	43.4 ± 0.7	42.8 ± 0.5	43.2 ± 0.5	43.5 ± 0.4	43.7 ± 0.3
Week 14	46.7 ± 0.3	46.2 ± 0.5	46.1 ± 0.2	47.2 ± 0.4	47.5 ± 0.6	48.1 ± 0.6
Hemoglobin (g/dL)						
Day 4	12.8 ± 0.1	12.6 ± 0.2	12.3 ± 0.1	12.5 ± 0.2	12.7 ± 0.1	12.9 ± 0.2
Day 23	14.4 ± 0.2	14.3 ± 0.2	14.2 ± 0.1	14.3 ± 0.1	14.4 ± 0.1	14.4 ± 0.1
Week 14	15.2 ± 0.1	15.0 ± 0.1	15.1 ± 0.1	15.3 ± 0.1	15.5 ± 0.1	15.8 ± 0.1*
Erythrocytes (10^6/µL)						
Day 4	6.54 ± 0.08	6.50 ± 0.09	6.27 ± 0.09	6.35 ± 0.09	6.52 ± 0.07	6.65 ± 0.09
Day 23	7.68 ± 0.11	7.66 ± 0.12	7.51 ± 0.10	7.61 ± 0.09	7.69 ± 0.09	7.75 ± 0.07
Week 14	9.09 ± 0.07	8.97 ± 0.10	9.04 ± 0.04	9.14 ± 0.07	9.25 ± 0.09	9.42 ± 0.09*
Reticulocytes (10^6/µL)						
Day 4	0.39 ± 0.04	0.32 ± 0.04	0.30 ± 0.04	0.28 ± 0.03	0.40 ± 0.03	0.31 ± 0.04
Day 23	0.17 ± 0.02	0.16 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.15 ± 0.01
Week 14	0.15 ± 0.01	0.14 ± 0.02	0.15 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
Nucleated erythrocytes (10^3/µL)						
Day 4	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.02	0.02 ± 0.02	0.00 ± 0.00	0.01 ± 0.01
Day 23	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Mean cell volume (fL)						
Day 4	59.7 ± 0.2	58.9 ± 0.3	59.1 ± 0.3	59.3 ± 0.3	59.2 ± 0.2	59.2 ± 0.2
Day 23	56.7 ± 0.3	56.6 ± 0.2	57.0 ± 0.3	56.7 ± 0.2	56.5 ± 0.3	56.4 ± 0.3
Week 14	51.4 ± 0.1	51.5 ± 0.2	51.0 ± 0.2	51.6 ± 0.1	51.4 ± 0.3	51.0 ± 0.3
Mean cell hemoglobin (pg)						
Day 4	19.5 ± 0.1	19.4 ± 0.1	19.6 ± 0.1	19.6 ± 0.1	19.5 ± 0.1	19.4 ± 0.1
Day 23	18.7 ± 0.1	18.7 ± 0.1	18.9 ± 0.1	18.8 ± 0.1	18.7 ± 0.1	18.6 ± 0.1
Week 14	16.7 ± 0.1	16.8 ± 0.1	16.7 ± 0.1	16.8 ± 0.1	16.7 ± 0.1	16.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.6 ± 0.1	32.9 ± 0.2	33.1 ± 0.1	33.0 ± 0.1	32.9 ± 0.1	32.8 ± 0.1
Day 23	33.1 ± 0.1	33.0 ± 0.1	33.2 ± 0.2	33.1 ± 0.1	33.0 ± 0.2	33.0 ± 0.1
Week 14	32.5 ± 0.1	32.5 ± 0.2	32.8 ± 0.1	32.5 ± 0.1	32.6 ± 0.2	32.9 ± 0.2
Platelets (10^3/µL)						
Day 4	929.4 ± 37.5	944.9 ± 24.1	963.8 ± 12.9	910.1 ± 19.3	951.4 ± 16.8	900.3 ± 36.4
Day 23	790.7 ± 13.6	791.8 ± 13.6	805.4 ± 6.4	781.4 ± 15.8	800.6 ± 13.1	820.3 ± 13.6
Week 14	708.6 ± 15.6	719.9 ± 11.7	689.5 ± 13.9	738.7 ± 17.6	706.2 ± 12.4	729.3 ± 14.9
Leukocytes (10^3/µL)						
Day 4	7.48 ± 0.33	7.15 ± 0.29	6.96 ± 0.48	6.67 ± 0.56	6.90 ± 0.25	8.12 ± 0.18
Day 23	7.37 ± 0.56	7.76 ± 0.61	7.68 ± 0.36	7.22 ± 0.54	7.83 ± 0.35	7.13 ± 0.51
Week 14	9.22 ± 0.28	9.64 ± 0.38	8.27 ± 0.40	8.42 ± 0.30	7.82 ± 0.29**	8.51 ± 0.49
Segmented neutrophils (10^3/µL)						
Day 4	1.14 ± 0.13	1.19 ± 0.10	1.06 ± 0.15	0.99 ± 0.12	0.87 ± 0.09	1.24 ± 0.14
Day 23	0.71 ± 0.08	0.88 ± 0.13	0.75 ± 0.09	0.86 ± 0.10	0.76 ± 0.07	0.91 ± 0.07
Week 14	1.16 ± 0.13	1.25 ± 0.09	1.33 ± 0.11	1.13 ± 0.08	1.28 ± 0.17	2.10 ± 0.19**

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	9	10	10
Day 23	9	10	10	9	10	10
Week 14	9	10	10	10	10	9
Bands (10^3/μL)						
Day 4	0.02 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	0.05 ± 0.02
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)						
Day 4	6.16 ± 0.32	5.70 ± 0.23	5.72 ± 0.40	5.52 ± 0.46	5.87 ± 0.23	6.63 ± 0.14
Day 23	6.57 ± 0.50	6.78 ± 0.54	6.84 ± 0.35	6.26 ± 0.48	6.97 ± 0.32	6.12 ± 0.51
Week 14	7.86 ± 0.23	8.19 ± 0.38	6.74 ± 0.34*	7.11 ± 0.28	6.40 ± 0.32**	6.25 ± 0.49**
Monocytes (10^3/μL)						
Day 4	0.15 ± 0.03	0.19 ± 0.06	0.13 ± 0.04	0.10 ± 0.04	0.12 ± 0.05	0.17 ± 0.03
Day 23	0.06 ± 0.02	0.08 ± 0.02	0.06 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
Week 14	0.16 ± 0.02	0.14 ± 0.02	0.14 ± 0.04	0.10 ± 0.03	0.09 ± 0.03	0.13 ± 0.04
Basophils (10^3/μL)						
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)						
Day 4	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
Day 23	0.03 ± 0.02	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
Week 14	0.04 ± 0.02	0.06 ± 0.02	0.07 ± 0.01	0.09 ± 0.03	0.06 ± 0.01	0.03 ± 0.02
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	9.6 ± 0.2	9.6 ± 0.4	10.4 ± 0.6	9.7 ± 0.4	9.7 ± 0.4	10.0 ± 0.3
Day 23	12.0 ± 0.5	11.6 ± 0.5	11.5 ± 0.4	12.4 ± 0.4	12.0 ± 0.4	11.3 ± 0.5
Week 14	16.5 ± 0.6	15.2 ± 0.6	16.6 ± 0.3	16.8 ± 0.5	16.6 ± 0.5	15.4 ± 0.3
Creatinine (mg/dL)						
Day 4	0.43 ± 0.02	0.39 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.40 ± 0.00	0.42 ± 0.01
Day 23	0.45 ± 0.02	0.43 ± 0.02	0.44 ± 0.02	0.47 ± 0.02	0.48 ± 0.01	0.48 ± 0.01
Week 14	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.02	0.51 ± 0.01	0.52 ± 0.01	0.51 ± 0.01
Total protein (g/dL)						
Day 4	5.4 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.4 ± 0.1
Day 23	5.8 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1
Week 14	6.5 ± 0.1	6.6 ± 0.1	6.5 ± 0.0	6.6 ± 0.0	6.6 ± 0.0	6.6 ± 0.0
Albumin (g/dL)						
Day 4	4.1 ± 0.0	4.0 ± 0.1	4.0 ± 0.0	4.1 ± 0.1	4.0 ± 0.0	4.1 ± 0.1
Day 23	4.3 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.3 ± 0.0	4.3 ± 0.0	4.2 ± 0.0
Week 14	4.6 ± 0.0	4.6 ± 0.0	4.6 ± 0.0	4.7 ± 0.0	4.6 ± 0.0	4.7 ± 0.0
Alanine aminotransferase (IU/L)						
Day 4	78 ± 2	78 ± 3	77 ± 2	78 ± 2	76 ± 4	83 ± 3
Day 23	66 ± 2	66 ± 1	62 ± 2	65 ± 2	61 ± 2	64 ± 1
Week 14	67 ± 2	67 ± 2	66 ± 3	73 ± 3	66 ± 2	79 ± 3

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Alkaline phosphatase (IU/L)						
Day 4	812 ± 21	790 ± 23	779 ± 17	782 ± 10	752 ± 12*	754 ± 9*
Day 23	502 ± 6	497 ± 10	480 ± 7	489 ± 13	489 ± 7	498 ± 8
Week 14	291 ± 6	276 ± 4	284 ± 7	262 ± 4**	279 ± 4	286 ± 7
Creatine kinase (IU/L)						
Day 4	266 ± 12	313 ± 43[b]	283 ± 19	406 ± 63[b]	359 ± 58	354 ± 36
Day 23	526 ± 83	334 ± 43[b]	526 ± 100	447 ± 49[b]	426 ± 50	446 ± 49
Week 14	179 ± 22	230 ± 41	183 ± 28	196 ± 25	251 ± 28	311 ± 73
Sorbitol dehydrogenase (IU/L)						
Day 4	13 ± 1	14 ± 1	13 ± 0	14 ± 1	14 ± 1	14 ± 1
Day 23	15 ± 1	15 ± 1	15 ± 1	15 ± 1	16 ± 1	15 ± 1
Week 14	18 ± 1	19 ± 1	18 ± 1	17 ± 1	18 ± 1	17 ± 1
Bile acids (μmol/L)						
Day 4	26.2 ± 2.6	24.8 ± 1.9	24.3 ± 1.6	24.2 ± 2.3	22.9 ± 1.6	26.1 ± 1.8
Day 23	31.8 ± 3.0	25.7 ± 1.8	29.3 ± 2.5	24.3 ± 2.3	25.4 ± 2.5	22.3 ± 2.4
Week 14	24.4 ± 2.8	23.3 ± 2.6	27.0 ± 3.5	28.1 ± 2.1	28.5 ± 2.8	24.9 ± 1.9

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Female						
Hematology						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	9	10	8	10
Hematocrit (%)						
Day 4	40.6 ± 0.6	40.9 ± 0.3	40.8 ± 0.7	39.9 ± 0.4	39.8 ± 0.7	40.3 ± 0.4
Day 23	45.7 ± 0.3	45.8 ± 0.4	46.8 ± 0.4	46.4 ± 0.5	46.5 ± 0.6	46.3 ± 0.7
Week 14	45.0 ± 0.3	45.9 ± 0.3	45.4 ± 0.4	44.6 ± 0.7	46.0 ± 0.4	45.7 ± 0.5
Hemoglobin (g/dL)						
Day 4	13.5 ± 0.2	13.5 ± 0.1	13.6 ± 0.2	13.3 ± 0.1	13.2 ± 0.2	13.4 ± 0.1
Day 23	14.9 ± 0.1	14.8 ± 0.1	15.2 ± 0.1	15.2 ± 0.1	15.2 ± 0.2	15.1 ± 0.2
Week 14	14.8 ± 0.1	15.0 ± 0.1	15.0 ± 0.1	14.7 ± 0.2	15.1 ± 0.1	15.2 ± 0.1**
Erythrocytes (10^6/µL)						
Day 4	6.85 ± 0.11	6.93 ± 0.06	6.90 ± 0.13	6.81 ± 0.08	6.81 ± 0.12	6.84 ± 0.08
Day 23	7.93 ± 0.06	7.91 ± 0.06	8.03 ± 0.07	8.05 ± 0.08	8.06 ± 0.12	8.02 ± 0.13
Week 14	8.22 ± 0.06	8.43 ± 0.06	8.34 ± 0.07	8.19 ± 0.15	8.42 ± 0.07	8.44 ± 0.08
Reticulocytes (10^6/µL)						
Day 4	0.36 ± 0.03	0.38 ± 0.02	0.34 ± 0.03	0.34 ± 0.03	0.34 ± 0.02	0.33 ± 0.02
Day 23	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.10 ± 0.01
Week 14	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
Nucleated erythrocytes (10^3/µL)						
Day 4	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Week 14	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Mean cell volume (fL)						
Day 4	59.3 ± 0.2	59.1 ± 0.3	59.2 ± 0.2	58.7 ± 0.4	58.4 ± 0.5	58.9 ± 0.2
Day 23	57.7 ± 0.3	57.9 ± 0.2	58.2 ± 0.2	57.6 ± 0.3	57.7 ± 0.4	57.7 ± 0.3
Week 14	54.7 ± 0.2	54.4 ± 0.3	54.4 ± 0.2	54.5 ± 0.3	54.6 ± 0.3	54.1 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.6 ± 0.1	19.5 ± 0.1	19.7 ± 0.1	19.6 ± 0.1	19.5 ± 0.1	19.6 ± 0.1
Day 23	18.8 ± 0.1	18.7 ± 0.1	18.9 ± 0.1	18.9 ± 0.1	18.9 ± 0.1	18.8 ± 0.1
Week 14	18.0 ± 0.1	17.9 ± 0.1	17.9 ± 0.1	18.0 ± 0.1	17.9 ± 0.1	18.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.2 ± 0.1	33.0 ± 0.1	33.2 ± 0.1	33.3 ± 0.1	33.3 ± 0.1	33.3 ± 0.1
Day 23	32.6 ± 0.1	32.4 ± 0.1	32.5 ± 0.1	32.7 ± 0.1	32.7 ± 0.1	32.6 ± 0.1
Week 14	32.9 ± 0.1	32.8 ± 0.1	33.0 ± 0.1	33.1 ± 0.1	32.8 ± 0.2	33.3 ± 0.2
Platelets (10^3/µL)						
Day 4	958.6 ± 16.1	892.6 ± 14.8	952.6 ± 21.9	990.1 ± 19.9	928.9 ± 17.5	941.5 ± 36.2
Day 23	804.6 ± 17.8	784.4 ± 24.2	794.9 ± 16.6	831.3 ± 16.3	796.5 ± 22.0	802.1 ± 12.0
Week 14	685.4 ± 19.0	667.8 ± 15.2	689.4 ± 11.8	677.9 ± 14.7	705.6 ± 12.2	711.2 ± 17.4
Leukocytes (10^3/µL)						
Day 4	7.39 ± 0.32	8.05 ± 0.28	8.04 ± 0.31	7.47 ± 0.41	7.78 ± 0.38	7.95 ± 0.27
Day 23	8.96 ± 0.60	9.39 ± 0.71	10.00 ± 0.84	9.52 ± 0.96	9.66 ± 0.76	10.26 ± 0.57
Week 14	8.89 ± 0.19	8.85 ± 0.62	8.80 ± 0.38	8.65 ± 0.37	9.58 ± 0.42	8.49 ± 0.52
Segmented neutrophils (10^3/µL)						
Day 4	1.07 ± 0.11	1.02 ± 0.12	0.99 ± 0.11	0.90 ± 0.07	1.00 ± 0.09	1.08 ± 0.08
Day 23	0.95 ± 0.09	0.85 ± 0.08[b]	0.90 ± 0.09	1.00 ± 0.17	0.90 ± 0.10	0.94 ± 0.10
Week 14	1.29 ± 0.08	1.38 ± 0.12	1.07 ± 0.05	1.26 ± 0.13	1.34 ± 0.11	1.71 ± 0.22

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Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	9	10	8	10
Bands ($10^3/\mu L$)						
Day 4	0.00 ± 0.00	0.00 ± 0.00[b]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes ($10^3/\mu L$)						
Day 4	6.19 ± 0.29	6.87 ± 0.31	6.86 ± 0.26	6.46 ± 0.38	6.65 ± 0.37	6.71 ± 0.25
Day 23	7.84 ± 0.56	8.38 ± 0.62	8.84 ± 0.78	8.37 ± 0.81	8.58 ± 0.65	9.15 ± 0.54
Week 14	7.35 ± 0.23	7.21 ± 0.52	7.59 ± 0.35	7.15 ± 0.35	7.97 ± 0.36	6.66 ± 0.33
Monocytes ($10^3/\mu L$)						
Day 4	0.11 ± 0.03	0.14 ± 0.02	0.15 ± 0.03	0.09 ± 0.03	0.06 ± 0.02	0.12 ± 0.02
Day 23	0.12 ± 0.03	0.13 ± 0.04	0.14 ± 0.02	0.11 ± 0.03	0.11 ± 0.02	0.13 ± 0.04
Week 14	0.15 ± 0.04	0.18 ± 0.03	0.09 ± 0.01	0.15 ± 0.05	0.18 ± 0.05	0.09 ± 0.03
Basophils ($10^3/\mu L$)						
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils ($10^3/\mu L$)						
Day 4	0.03 ± 0.01	0.03 ± 0.02	0.05 ± 0.02	0.02 ± 0.01	0.07 ± 0.02	0.04 ± 0.02
Day 23	0.05 ± 0.02	0.07 ± 0.02	0.11 ± 0.04	0.04 ± 0.02	0.07 ± 0.03	0.04 ± 0.02
Week 14	0.11 ± 0.02	0.08 ± 0.02	0.05 ± 0.02	0.09 ± 0.03	0.10 ± 0.04	0.03 ± 0.02
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	11.4 ± 0.5	11.6 ± 0.4	12.1 ± 0.6	11.5 ± 0.4	11.7 ± 0.5	11.3 ± 0.4
Day 23	15.3 ± 0.3	14.0 ± 0.3	14.9 ± 0.4	15.0 ± 0.4	14.7 ± 0.4	13.8 ± 0.6
Week 14	17.5 ± 0.3	18.6 ± 0.5	18.8 ± 0.6	17.6 ± 0.9	19.1 ± 0.5	16.7 ± 0.4
Creatinine (mg/dL)						
Day 4	0.42 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.43 ± 0.02
Day 23	0.39 ± 0.01	0.40 ± 0.00	0.40 ± 0.00	0.42 ± 0.01	0.42 ± 0.02	0.40 ± 0.00
Week 14	0.53 ± 0.02	0.52 ± 0.01	0.52 ± 0.01	0.53 ± 0.02	0.54 ± 0.02	0.52 ± 0.01
Total protein (g/dL)						
Day 4	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.6 ± 0.1
Day 23	5.6 ± 0.1	5.5 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1
Week 14	6.4 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.5 ± 0.1
Albumin (g/dL)						
Day 4	4.3 ± 0.0	4.3 ± 0.1	4.3 ± 0.0	4.3 ± 0.0	4.2 ± 0.1	4.3 ± 0.0
Day 23	4.3 ± 0.0	4.2 ± 0.0	4.3 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.4 ± 0.1
Week 14	4.6 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.7 ± 0.0	4.7 ± 0.0
Alanine aminotransferase (IU/L)						
Day 4	67 ± 3	67 ± 2	64 ± 2	62 ± 1	65 ± 2	63 ± 3
Day 23	59 ± 3	57 ± 2	57 ± 1	63 ± 3	58 ± 1	59 ± 2
Week 14	92 ± 5	90 ± 5	103 ± 11	85 ± 3	85 ± 5	80 ± 4

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Female (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Alkaline phosphatase (IU/L)						
Day 4	645 ± 22	661 ± 15	616 ± 18	631 ± 17	637 ± 19	622 ± 18
Day 23	421 ± 9	405 ± 9	423 ± 7	426 ± 10	431 ± 14	435 ± 10
Week 14	288 ± 6	300 ± 8	278 ± 6	265 ± 11	280 ± 8	256 ± 7**
Creatine kinase (IU/L)						
Day 4	262 ± 17	267 ± 22	299 ± 24	330 ± 68	290 ± 31	260 ± 14
Day 23	360 ± 39	287 ± 33	353 ± 64	326 ± 36	334 ± 25	331 ± 44
Week 14	193 ± 32	150 ± 15	180 ± 33	160 ± 20	218 ± 43	184 ± 28
Sorbitol dehydrogenase (IU/L)						
Day 4	12 ± 0	12 ± 0	12 ± 0	12 ± 0	12 ± 0	12 ± 0
Day 23	15 ± 1	15 ± 1	16 ± 1	18 ± 2	17 ± 1	17 ± 1
Week 14	24 ± 2	22 ± 2	27 ± 5	23 ± 1	23 ± 1	20 ± 1
Bile acids (μmol/L)						
Day 4	19.9 ± 1.1	20.4 ± 1.5	18.9 ± 1.2	18.9 ± 1.4	18.8 ± 1.4	17.2 ± 1.1
Day 23	19.3 ± 2.1	25.1 ± 2.2	23.9 ± 2.5	18.9 ± 2.3	21.8 ± 1.7	21.9 ± 2.2
Week 14	22.3 ± 3.2	30.9 ± 3.8	29.3 ± 5.7	25.8 ± 2.5	26.7 ± 2.4	24.6 ± 1.8

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9

TABLE E2
Hematology Data for B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	10	10	10
Male						
Hematocrit (%)	47.8 ± 1.1	46.4 ± 1.0	45.5 ± 0.6	46.2 ± 0.6	45.4 ± 0.5	44.9 ± 0.4
Hemoglobin (g/dL)	16.2 ± 0.3	15.8 ± 0.3	15.5 ± 0.2	15.8 ± 0.2	15.4 ± 0.1	15.3 ± 0.1
Erythrocytes (10^6/μL)	10.40 ± 0.24	10.14 ± 0.22	9.89 ± 0.15	10.10 ± 0.15	9.90 ± 0.12	9.85 ± 0.08
Reticulocytes (10^6/μL)	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.11 ± 0.01	0.06 ± 0.01	0.08 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	45.9 ± 0.1	45.8 ± 0.2	46.1 ± 0.2	45.7 ± 0.2	45.9 ± 0.2	45.6 ± 0.2
Mean cell hemoglobin (pg)	15.6 ± 0.1	15.5 ± 0.1	15.7 ± 0.1	15.6 ± 0.0	15.6 ± 0.1	15.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.9 ± 0.2	34.0 ± 0.1	34.0 ± 0.2	34.1 ± 0.1	33.9 ± 0.1	34.0 ± 0.1
Platelets (10^3/μL)	793.8 ± 13.4	840.6 ± 24.7	783.9 ± 17.6	858.3 ± 32.9	857.6 ± 40.1	860.6 ± 24.2
Leukocytes (10^3/μL)	3.94 ± 0.14	4.39 ± 0.22	3.89 ± 0.39	4.08 ± 0.21	4.46 ± 0.26	4.86 ± 0.28
Segmented neutrophils (10^3/μL)	0.58 ± 0.06	0.72 ± 0.09	0.53 ± 0.06	0.53 ± 0.03	0.66 ± 0.04	0.88 ± 0.07*
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10^3/μL)	0.08 ± 0.02	0.08 ± 0.01	0.10 ± 0.02	0.11 ± 0.01	0.09 ± 0.02	0.10 ± 0.02
Lymphocytes (10^3/μL)	3.19 ± 0.14	3.54 ± 0.13	3.17 ± 0.30	3.38 ± 0.20	3.64 ± 0.24	3.79 ± 0.22
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.08 ± 0.02	0.06 ± 0.01	0.09 ± 0.04	0.06 ± 0.01	0.07 ± 0.02	0.09 ± 0.02
Female						
Hematocrit (%)	49.8 ± 1.3	50.2 ± 0.8	49.6 ± 0.9	48.9 ± 0.7	48.9 ± 0.7	48.7 ± 0.8
Hemoglobin (g/dL)	16.4 ± 0.4	16.5 ± 0.3	16.2 ± 0.3	16.0 ± 0.2	16.0 ± 0.2	15.9 ± 0.3
Erythrocytes (10^6/μL)	10.53 ± 0.29	10.51 ± 0.16	10.44 ± 0.21	10.28 ± 0.15	10.20 ± 0.14	10.19 ± 0.18
Reticulocytes (10^6/μL)	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00
Mean cell volume (fL)	47.4 ± 0.1	47.7 ± 0.1	47.5 ± 0.2	47.6 ± 0.1	47.9 ± 0.1	47.8 ± 0.2
Mean cell hemoglobin (pg)	15.6 ± 0.1	15.7 ± 0.1	15.5 ± 0.1	15.5 ± 0.1	15.7 ± 0.1	15.6 ± 0.0
Mean cell hemoglobin concentration (g/dL)	32.9 ± 0.2	32.8 ± 0.2	32.6 ± 0.1	32.7 ± 0.2	32.9 ± 0.2	32.6 ± 0.1
Platelets (10^3/μL)	753.8 ± 46.5	816.3 ± 21.1	785.3 ± 32.8	804.2 ± 27.3	750.6 ± 43.9	856.2 ± 35.0
Leukocytes (10^3/μL)	5.55 ± 0.42	6.00 ± 0.33	5.52 ± 0.23	5.44 ± 0.37	6.33 ± 0.46	5.83 ± 0.20
Segmented neutrophils (10^3/μL)	0.66 ± 0.10	0.64 ± 0.07	0.53 ± 0.05	0.73 ± 0.09	1.10 ± 0.15*	1.11 ± 0.21*
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	4.78 ± 0.40	5.28 ± 0.29	4.88 ± 0.20	4.57 ± 0.28	5.09 ± 0.33	4.57 ± 0.13
Monocytes (10^3/μL)	0.05 ± 0.02	0.04 ± 0.02	0.05 ± 0.01	0.08 ± 0.03	0.08 ± 0.03	0.08 ± 0.01
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.06 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.02

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.

APPENDIX F
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	170 ± 6	162 ± 6	167 ± 4	163 ± 8	163 ± 3	155 ± 4
Heart						
Absolute	0.665 ± 0.017	0.619 ± 0.023	0.651 ± 0.016	0.640 ± 0.022	0.656 ± 0.023	0.707 ± 0.042
Relative	3.928 ± 0.054	3.814 ± 0.063	3.902 ± 0.059	3.939 ± 0.100	4.024 ± 0.101	4.548 ± 0.169**
R. Kidney						
Absolute	0.688 ± 0.023	0.642 ± 0.033	0.697 ± 0.027	0.692 ± 0.046	0.713 ± 0.035	0.706 ± 0.021
Relative	4.055 ± 0.046	3.945 ± 0.058	4.176 ± 0.078	4.235 ± 0.117	4.370 ± 0.131*	4.555 ± 0.047**
Liver						
Absolute	8.298 ± 0.442	7.700 ± 0.567	8.168 ± 0.257	8.065 ± 0.498	8.256 ± 0.164	7.806 ± 0.292
Relative	48.8 ± 1.1	47.2 ± 1.8	48.9 ± 0.6	49.4 ± 1.1	50.7 ± 0.8	50.3 ± 0.8
Lung						
Absolute	1.208 ± 0.059	1.133 ± 0.063	1.163 ± 0.089	1.225 ± 0.178	1.281 ± 0.105	1.163 ± 0.043
Relative	7.119 ± 0.233	6.981 ± 0.327	6.964 ± 0.458	7.537 ± 1.066	7.880 ± 0.685	7.501 ± 0.155
R. Testis						
Absolute	1.059 ± 0.026	1.062 ± 0.036	1.069 ± 0.026	1.035 ± 0.039	1.068 ± 0.025	1.095 ± 0.030
Relative	6.251 ± 0.059	6.551 ± 0.147	6.408 ± 0.069	6.362 ± 0.071	6.559 ± 0.064*	7.066 ± 0.076**
Thymus						
Absolute	0.440 ± 0.014	0.373 ± 0.021*	0.403 ± 0.027	0.384 ± 0.005	0.404 ± 0.017	0.360 ± 0.008*
Relative	2.600 ± 0.088	2.302 ± 0.123	2.408 ± 0.117	2.374 ± 0.104	2.482 ± 0.096	2.327 ± 0.063
Female						
Necropsy body wt	124 ± 3	124 ± 3	122 ± 1	122 ± 2	128 ± 5	118 ± 2
Heart						
Absolute	0.548 ± 0.036	0.552 ± 0.006	0.508 ± 0.006	0.526 ± 0.016	0.566 ± 0.036	0.517 ± 0.012
Relative	4.451 ± 0.352	4.453 ± 0.083	4.183 ± 0.036	4.321 ± 0.113	4.398 ± 0.133	4.388 ± 0.125
R. Kidney						
Absolute	0.536 ± 0.013	0.552 ± 0.023	0.546 ± 0.012	0.567 ± 0.022	0.609 ± 0.028	0.570 ± 0.020
Relative	4.329 ± 0.039	4.437 ± 0.096	4.495 ± 0.079	4.656 ± 0.126*	4.738 ± 0.098**	4.828 ± 0.104**
Liver						
Absolute	5.972 ± 0.137	5.908 ± 0.153	5.907 ± 0.154	5.994 ± 0.101	6.491 ± 0.532	5.802 ± 0.211
Relative	48.4 ± 1.6	47.6 ± 0.6	48.6 ± 0.9	49.3 ± 0.8	50.3 ± 2.3	49.2 ± 1.1
Lung						
Absolute	1.005 ± 0.050	1.018 ± 0.060	0.952 ± 0.055	0.973 ± 0.043	1.018 ± 0.049	0.860 ± 0.026
Relative	8.105 ± 0.285	8.192 ± 0.388	7.828 ± 0.426	8.010 ± 0.424	7.940 ± 0.335	7.296 ± 0.202
Thymus						
Absolute	0.337 ± 0.011	0.365 ± 0.016	0.324 ± 0.024	0.342 ± 0.010	0.350 ± 0.020	0.325 ± 0.013
Relative	2.729 ± 0.124	2.937 ± 0.085	2.666 ± 0.198	2.812 ± 0.074	2.718 ± 0.063	2.762 ± 0.127

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test

** $P \leq 0.01$

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	280 ± 6	298 ± 6	282 ± 7	279 ± 6	272 ± 6	259 ± 7*
Heart						
Absolute	0.895 ± 0.020	0.940 ± 0.025	0.908 ± 0.026	0.904 ± 0.020	0.899 ± 0.019	0.882 ± 0.034
Relative	3.194 ± 0.028	3.157 ± 0.077	3.218 ± 0.053	3.244 ± 0.083	3.306 ± 0.053	3.397 ± 0.064
R. Kidney						
Absolute	1.010 ± 0.028	1.039 ± 0.027	0.989 ± 0.024	1.007 ± 0.019	0.995 ± 0.027	1.004 ± 0.034
Relative	3.603 ± 0.055	3.484 ± 0.047	3.507 ± 0.051	3.607 ± 0.043	3.653 ± 0.042	3.865 ± 0.053**
Liver						
Absolute	11.741 ± 0.345	12.165 ± 0.425	11.265 ± 0.351	11.115 ± 0.382	10.585 ± 0.436	10.607 ± 0.509
Relative	41.9 ± 0.7	40.8 ± 1.1	39.9 ± 0.7	39.7 ± 0.7	38.8 ± 1.0	40.7 ± 1.2
Lung						
Absolute	1.801 ± 0.117	1.716 ± 0.134	1.586 ± 0.071[b]	1.536 ± 0.074	1.605 ± 0.111	1.443 ± 0.049*
Relative	6.396 ± 0.311	5.747 ± 0.411	5.534 ± 0.206[b]	5.513 ± 0.280	5.918 ± 0.439	5.589 ± 0.212
R. Testis						
Absolute	1.402 ± 0.015	1.447 ± 0.023	1.382 ± 0.027	1.403 ± 0.015	1.402 ± 0.022	1.335 ± 0.026
Relative	5.020 ± 0.092	4.866 ± 0.091	4.905 ± 0.077	5.030 ± 0.068	5.161 ± 0.067	5.162 ± 0.083
Thymus						
Absolute	0.344 ± 0.010	0.366 ± 0.011	0.334 ± 0.016	0.320 ± 0.015	0.318 ± 0.014	0.273 ± 0.012**
Relative	1.230 ± 0.033	1.230 ± 0.036	1.177 ± 0.039	1.145 ± 0.051	1.170 ± 0.049	1.055 ± 0.044**
Female						
Necropsy body wt	177 ± 3	169 ± 2	174 ± 2	179 ± 4	167 ± 2	171 ± 4
Heart						
Absolute	0.662 ± 0.015	0.613 ± 0.009*	0.643 ± 0.018	0.649 ± 0.010	0.610 ± 0.011*	0.662 ± 0.010
Relative	3.748 ± 0.047	3.636 ± 0.063	3.690 ± 0.092	3.639 ± 0.053	3.652 ± 0.051	3.879 ± 0.063
R. Kidney						
Absolute	0.681 ± 0.012	0.639 ± 0.013	0.690 ± 0.011	0.709 ± 0.022	0.666 ± 0.016	0.716 ± 0.023
Relative	3.860 ± 0.049	3.787 ± 0.058	3.964 ± 0.079	3.960 ± 0.053	3.985 ± 0.065	4.197 ± 0.149**
Liver						
Absolute	6.560 ± 0.076	6.215 ± 0.116	6.562 ± 0.225	6.793 ± 0.325	6.383 ± 0.102	6.374 ± 0.223
Relative	37.2 ± 0.5	36.8 ± 0.4	37.7 ± 1.3	37.8 ± 1.0	38.2 ± 0.5	37.2 ± 0.8
Lung						
Absolute	1.112 ± 0.038	1.100 ± 0.033	1.178 ± 0.079	1.154 ± 0.054	1.196 ± 0.028	1.191 ± 0.052
Relative	6.284 ± 0.127	6.528 ± 0.212	6.737 ± 0.400	6.461 ± 0.290	7.163 ± 0.172	6.935 ± 0.190
Thymus						
Absolute	0.281 ± 0.009	0.227 ± 0.006*	0.278 ± 0.008	0.265 ± 0.009	0.241 ± 0.006**	0.237 ± 0.007**
Relative	1.597 ± 0.065	1.343 ± 0.036**	1.598 ± 0.061	1.486 ± 0.056	1.442 ± 0.034	1.385 ± 0.042*

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 2-Week Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	25.2 ± 0.5	25.6 ± 0.3	25.0 ± 0.4	25.5 ± 0.6	23.7 ± 1.5	23.1 ± 1.3
Heart						
Absolute	0.129 ± 0.001	0.137 ± 0.002	0.143 ± 0.009	0.144 ± 0.004	0.135 ± 0.008	0.125 ± 0.007
Relative	5.113 ± 0.091	5.368 ± 0.083	5.710 ± 0.286*	5.621 ± 0.071	5.716 ± 0.114*	5.413 ± 0.073
R. Kidney						
Absolute	0.240 ± 0.008	0.248 ± 0.009	0.242 ± 0.009	0.261 ± 0.010	0.229 ± 0.013	0.238 ± 0.010
Relative	9.511 ± 0.283	9.691 ± 0.306	9.646 ± 0.212	10.208 ± 0.284	9.712 ± 0.219	10.382 ± 0.249
Liver						
Absolute	1.409 ± 0.027	1.427 ± 0.059	1.392 ± 0.038	1.462 ± 0.031	1.240 ± 0.133	1.250 ± 0.120
Relative	55.9 ± 0.8	55.8 ± 1.8	55.6 ± 0.7	57.3 ± 1.0	51.7 ± 3.0	53.7 ± 2.8
Lung						
Absolute	0.202 ± 0.004	0.191 ± 0.004	0.193 ± 0.014	0.211 ± 0.006	0.182 ± 0.009	0.180 ± 0.008
Relative	8.009 ± 0.199	7.487 ± 0.148	7.702 ± 0.461	8.266 ± 0.269	7.720 ± 0.159	7.938 ± 0.652
R. Testis						
Absolute	0.099 ± 0.002	0.100 ± 0.004	0.104 ± 0.004	0.104 ± 0.002	0.101 ± 0.002	0.100 ± 0.002
Relative	3.942 ± 0.057	3.924 ± 0.179	4.146 ± 0.105	4.057 ± 0.060	4.347 ± 0.270	4.436 ± 0.371
Thymus						
Absolute	0.060 ± 0.003	0.049 ± 0.004	0.054 ± 0.003	0.045 ± 0.003**	0.038 ± 0.003**	0.036 ± 0.005**
Relative	2.365 ± 0.119	1.922 ± 0.143	2.148 ± 0.105	1.747 ± 0.104**	1.615 ± 0.090**	1.532 ± 0.185**
Female						
Necropsy body wt	21.0 ± 0.5	21.3 ± 0.5	22.0 ± 0.3	21.5 ± 0.2	22.5 ± 0.3*	22.4 ± 0.4*
Heart						
Absolute	0.121 ± 0.004	0.124 ± 0.002	0.128 ± 0.003	0.132 ± 0.003	0.126 ± 0.005	0.132 ± 0.003
Relative	5.747 ± 0.133	5.842 ± 0.135	5.840 ± 0.201	6.148 ± 0.149	5.590 ± 0.179	5.911 ± 0.164
R. Kidney						
Absolute	0.176 ± 0.008	0.171 ± 0.004	0.181 ± 0.003	0.176 ± 0.003	0.189 ± 0.007	0.191 ± 0.008
Relative	8.358 ± 0.245	8.048 ± 0.124	8.239 ± 0.208	8.186 ± 0.136	8.390 ± 0.237	8.511 ± 0.248
Liver						
Absolute	1.282 ± 0.044	1.246 ± 0.056	1.346 ± 0.047	1.297 ± 0.036	1.295 ± 0.038	1.316 ± 0.034
Relative	61.0 ± 0.9	58.5 ± 1.3	61.0 ± 1.2	60.4 ± 1.2	57.6 ± 1.2	58.7 ± 1.0
Lung						
Absolute	0.176 ± 0.014	0.194 ± 0.009	0.183 ± 0.011	0.176 ± 0.002	0.197 ± 0.008	0.178 ± 0.005
Relative	8.327 ± 0.511	9.119 ± 0.331	8.309 ± 0.501	8.188 ± 0.133	8.777 ± 0.264	7.927 ± 0.234
Thymus						
Absolute	0.073 ± 0.007	0.073 ± 0.003	0.082 ± 0.006	0.073 ± 0.002	0.076 ± 0.003	0.078 ± 0.006
Relative	3.488 ± 0.300	3.420 ± 0.060	3.718 ± 0.224	3.417 ± 0.074	3.369 ± 0.155	3.453 ± 0.219

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	36.70 ± 0.70	34.86 ± 0.81	36.80 ± 1.13	36.10 ± 0.89	34.87 ± 0.70	34.97 ± 0.70
Heart						
Absolute	0.180 ± 0.004	0.167 ± 0.003	0.184 ± 0.005	0.178 ± 0.007	0.186 ± 0.009	0.180 ± 0.006
Relative	4.897 ± 0.087	4.818 ± 0.114	5.019 ± 0.137	4.941 ± 0.180	5.323 ± 0.215	5.137 ± 0.169
R. Kidney						
Absolute	0.334 ± 0.006	0.329 ± 0.008	0.349 ± 0.009	0.324 ± 0.010	0.331 ± 0.007	0.329 ± 0.008
Relative	9.117 ± 0.196	9.469 ± 0.267	9.518 ± 0.162	9.003 ± 0.272	9.528 ± 0.240	9.426 ± 0.213
Liver						
Absolute	1.744 ± 0.042	1.671 ± 0.052	1.865 ± 0.050	1.741 ± 0.068	1.748 ± 0.036	1.738 ± 0.039
Relative	47.6 ± 1.0	47.9 ± 1.0	50.8 ± 0.9	48.2 ± 1.3	50.2 ± 0.9	49.7 ± 0.7
Lung						
Absolute	0.302 ± 0.019	0.280 ± 0.009	0.281 ± 0.014	0.283 ± 0.018[b]	0.266 ± 0.013	0.267 ± 0.016
Relative	8.263 ± 0.538	8.044 ± 0.282	7.649 ± 0.340	7.848 ± 0.482[b]	7.656 ± 0.396	7.618 ± 0.402
R. Testis						
Absolute	0.112 ± 0.004	0.117 ± 0.003	0.119 ± 0.003	0.118 ± 0.004	0.115 ± 0.003	0.120 ± 0.003
Relative	3.058 ± 0.102	3.374 ± 0.105	3.250 ± 0.068	3.271 ± 0.105	3.304 ± 0.066	3.448 ± 0.104*
Thymus						
Absolute	0.043 ± 0.002	0.040 ± 0.004	0.042 ± 0.003	0.042 ± 0.002	0.041 ± 0.002	0.041 ± 0.002
Relative	1.168 ± 0.058	1.139 ± 0.110	1.128 ± 0.065	1.173 ± 0.051	1.168 ± 0.057	1.158 ± 0.037
Female						
Necropsy body wt	31.07 ± 1.36	31.88 ± 0.92	33.29 ± 1.65	32.85 ± 1.25	30.55 ± 0.54	31.05 ± 1.20
Heart						
Absolute	0.146 ± 0.004	0.161 ± 0.007	0.162 ± 0.005	0.151 ± 0.003	0.154 ± 0.003	0.161 ± 0.006
Relative	4.752 ± 0.157	5.083 ± 0.267	4.964 ± 0.278	4.648 ± 0.185	5.058 ± 0.138	5.247 ± 0.284
R. Kidney						
Absolute	0.216 ± 0.005	0.225 ± 0.004	0.226 ± 0.006	0.219 ± 0.004	0.221 ± 0.006	0.231 ± 0.006
Relative	7.043 ± 0.252	7.094 ± 0.173	6.880 ± 0.221	6.730 ± 0.223	7.234 ± 0.178	7.510 ± 0.265
Liver						
Absolute	1.521 ± 0.050	1.528 ± 0.051	1.629 ± 0.066	1.559 ± 0.035	1.527 ± 0.025	1.550 ± 0.046
Relative	49.4 ± 1.8	48.0 ± 1.2	49.2 ± 1.5	47.8 ± 1.4	50.0 ± 0.8	50.2 ± 1.4
Lung						
Absolute	0.301 ± 0.019	0.317 ± 0.012	0.296 ± 0.013	0.290 ± 0.013	0.311 ± 0.020	0.305 ± 0.018
Relative	9.724 ± 0.583	10.013 ± 0.502	9.069 ± 0.576	8.924 ± 0.474	10.196 ± 0.681	9.971 ± 0.712
Thymus						
Absolute	0.060 ± 0.003	0.060 ± 0.003	0.062 ± 0.005	0.058 ± 0.003	0.054 ± 0.003	0.059 ± 0.003
Relative	1.949 ± 0.088	1.879 ± 0.054	1.843 ± 0.101	1.769 ± 0.096	1.773 ± 0.085	1.895 ± 0.067

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

[b] n=9

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
n	14	15	12	14	13	11
Necropsy body wt	33.4 ± 0.8	32.5 ± 1.3	36.8 ± 1.3	34.7 ± 0.9	33.1 ± 0.9	35.8 ± 1.7
Heart						
Absolute	0.222 ± 0.004	0.211 ± 0.006	0.233 ± 0.009	0.225 ± 0.005	0.221 ± 0.006	0.289 ± 0.014**
Relative	6.700 ± 0.187	6.599 ± 0.260	6.389 ± 0.275	6.516 ± 0.113	6.678 ± 0.166	8.189 ± 0.411**
R. Kidney						
Absolute	0.299 ± 0.008	0.301 ± 0.018	0.306 ± 0.005	0.294 ± 0.006	0.300 ± 0.008	0.334 ± 0.010
Relative	8.977 ± 0.170	9.292 ± 0.415	8.381 ± 0.187	8.509 ± 0.168	9.079 ± 0.157	9.465 ± 0.321
Liver						
Absolute	1.833 ± 0.049	1.778 ± 0.075	1.920 ± 0.062	1.874 ± 0.055	1.800 ± 0.049	2.088 ± 0.081*
Relative	54.9 ± 0.9	54.8 ± 0.9	52.3 ± 1.1	54.2 ± 1.2	54.4 ± 0.9	58.7 ± 1.4
Lung						
Absolute	0.360 ± 0.013	0.315 ± 0.019	0.346 ± 0.013	0.345 ± 0.009	0.311 ± 0.013*	0.272 ± 0.016**
Relative	10.836 ± 0.405	9.715 ± 0.451	9.567 ± 0.486	10.007 ± 0.272	9.432 ± 0.411*	7.780 ± 0.613**
R. Testis						
Absolute	0.082 ± 0.005	0.089 ± 0.005	0.088 ± 0.002	0.091 ± 0.003	0.088 ± 0.003	0.075 ± 0.003
Relative	2.468 ± 0.148	2.702 ± 0.116	2.409 ± 0.093	2.631 ± 0.097	2.678 ± 0.102	2.114 ± 0.099
Thymus						
Absolute	0.030 ± 0.002	0.025 ± 0.002	0.035 ± 0.004	0.033 ± 0.002	0.032 ± 0.004	0.023 ± 0.003
Relative	0.883 ± 0.071	0.765 ± 0.054	0.969 ± 0.107	0.950 ± 0.060	0.953 ± 0.088	0.636 ± 0.084
Female						
n	15	14	12	14	14	12
Necropsy body wt	28.4 ± 0.9	27.4 ± 0.7	28.8 ± 0.5	27.8 ± 0.4	27.0 ± 1.1	29.0 ± 0.7
Heart						
Absolute	0.195 ± 0.004	0.189 ± 0.003	0.187 ± 0.006	0.183 ± 0.005	0.200 ± 0.006	0.226 ± 0.008**
Relative	6.910 ± 0.193	6.932 ± 0.158	6.499 ± 0.185	6.582 ± 0.148	7.626 ± 0.561	7.808 ± 0.284
R. Kidney						
Absolute	0.215 ± 0.004	0.210 ± 0.004	0.217 ± 0.005	0.209 ± 0.004	0.204 ± 0.006	0.248 ± 0.007**
Relative	7.610 ± 0.169	7.683 ± 0.144	7.573 ± 0.245	7.521 ± 0.137	7.667 ± 0.248	8.573 ± 0.252**
Liver						
Absolute	1.590 ± 0.042	1.550 ± 0.040	1.608 ± 0.036	1.515 ± 0.026[b]	1.482 ± 0.064	1.903 ± 0.087**
Relative	56.2 ± 1.1	56.6 ± 1.2	56.0 ± 1.8	54.5 ± 0.6[b]	55.1 ± 1.6	65.8 ± 2.9**
Lung						
Absolute	0.331 ± 0.007	0.321 ± 0.008	0.320 ± 0.012	0.308 ± 0.009[b]	0.301 ± 0.011*	0.246 ± 0.009**
Relative	11.757 ± 0.387	11.750 ± 0.329	11.109 ± 0.434	11.098 ± 0.341[b]	11.279 ± 0.389	8.548 ± 0.389**
Thymus						
Absolute	0.034 ± 0.002	0.034 ± 0.003	0.036 ± 0.003	0.033 ± 0.002	0.033 ± 0.002	0.025 ± 0.002
Relative	1.181 ± 0.046	1.247 ± 0.083	1.247 ± 0.117	1.165 ± 0.074	1.233 ± 0.080	0.881 ± 0.073*

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=13

APPENDIX G
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE G1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	280 ± 6	279 ± 6	272 ± 6	259 ± 7
L. Cauda epididymis	0.151 ± 0.005	0.135 ± 0.003	0.142 ± 0.005	0.150 ± 0.007
L. Epididymis	0.441 ± 0.010	0.429 ± 0.007	0.433 ± 0.011	0.436 ± 0.013
L. Testis	1.45 ± 0.02	1.46 ± 0.02	1.45 ± 0.03	1.36 ± 0.03*
Spermatid measurements				
Spermatid heads (10^7/g testis)	153 ± 5	147 ± 4	144 ± 4	161 ± 7
Spermatid heads (10^7/testis)	191 ± 7	186 ± 6	181 ± 7	189 ± 7
Spermatid heads (10^7/g cauda)	553 ± 25	588 ± 39	569 ± 31	549 ± 30
Spermatid heads (10^7/cauda)	83.1 ± 3.0	78.9 ± 5.1	80.5 ± 5.1	81.4 ± 3.7
Epididymal spermatozoal measurements				
Motility (%)	73.8 ± 1.4	75.1 ± 0.9	75.0 ± 1.2	74.7 ± 0.7

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test.
[a] Data are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE G2
Estrous Cycle Characterization for Female Rats in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	10
Necropsy body wt (g)	177 ± 3	179 ± 4	167 ± 2	171 ± 3
Estrous cycle length (days)	4.89 ± 0.07[b]	5.06 ± 0.15[c]	5.20 ± 0.20	5.20 ± 0.20
Estrous stages (% of cycle)				
Diestrus	42.5	45.0	45.0	50.0
Proestrus	13.3	15.8	17.5	16.7
Estrus	19.2	17.5	20.8	19.2
Metestrus	17.5	15.0	16.7	14.2
Uncertain diagnoses	7.5	6.7	0.0	0.0

[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight and estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.
[c] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.

TABLE G3
Summary of Reproductive Tissue Evaluations for Male B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	36.7 ± 0.7	36.1 ± 0.9	34.9 ± 0.7	35.0 ± 0.7
L. Cauda epididymis	0.015 ± 0.001	0.014 ± 0.001	0.014 ± 0.001	0.014 ± 0.001
L. Epididymis	0.040 ± 0.001	0.040 ± 0.001	0.040 ± 0.001	0.041 ± 0.001
L. Testis	0.11 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.11 ± 0.00
Spermatid measurements				
Spermatid heads (10^7/g testis)	231 ± 11	220 ± 8	248 ± 17	243 ± 11
Spermatid heads (10^7/testis)	21 ± 2	21 ± 1	23 ± 2	24 ± 1
Spermatid heads (10^7/g cauda)	986 ± 129	1,049 ± 57	1,135 ± 116	989 ± 53
Spermatid heads (10^7/cauda)	14.6 ± 2.1	14.1 ± 0.5	15.4 ± 1.2	14.3 ± 0.9
Epididymal spermatozoal measurements				
Motility (%)	73.7 ± 1.0	74.6 ± 1.4	75.1 ± 1.5	74.4 ± 1.0

[a] Data are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE G4
Estrous Cycle Characterization for Female B6C3F$_1$ Mice in the 3-Month Dermal Study of Trimethylolpropane Triacrylate[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	10
Necropsy body wt (g)	31.1 ± 1.4	32.9 ± 1.2	30.6 ± 0.5	31.1 ± 1.2
Estrous cycle length (days)	4.11 ± 0.18[b]	4.06 ± 0.06[b]	3.99 ± 0.15	4.47 ± 0.30
Estrous stages[c] (% of cycle)				
Diestrus	36.7	32.5	48.3	40.0
Proestrus	17.5	15.0	5.8	6.7
Estrus	23.3	30.8	26.7	32.5
Metestrus	20.0	21.7	12.5	19.2
Uncertain diagnoses	2.5	0.0	6.7	1.7

[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight and estrous cycle length).
[b] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.
[c] Evidence shows that females dosed with 6 or 12 mg/kg differ significantly (Wilk's Criterion, $P \leq 0.05$) from the vehicle control females in the relative length of time spent in the estrous stages.

APPENDIX H
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Trimethylolpropane Triacrylate

Trimethylolpropane triacrylate was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (01031AW), which was used throughout the studies. Identity, moisture content, purity, and stability analyses were conducted by the analytical chemistry laboratories and the study laboratory (Battelle Columbus Laboratories, Columbus, OH). Reports on analyses performed in support of the trimethylolpropane triacrylate studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a colorless to yellow viscous liquid, was identified as trimethylolpropane triacrylate by the analytical chemistry laboratory using infrared spectrometry and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using infrared spectrometry. All spectra were consistent with the structure of trimethylolpropane triacrylate; the infrared spectrum was also consistent with a literature spectrum (*Aldrich*, 1985). The infrared and NMR spectra are presented in Figures H1 through H3.

The analytical chemistry laboratories and the study laboratory determined moisture content using Karl Fischer titration and purity using elemental analyses, gas chromatography (GC), high-performance liquid chromatography (HPLC), and HPLC with mass spectrometry (HPLC/MS). GC was performed using systems A and B (Table H1). HPLC was performed with an Ultracarb 5 ODS (30) column (150 mm × 4.6 mm, 5-µm particle size; Phenomenex, Torrance, CA) and ultraviolet detection at 221 nm. A mobile phase of (A) methanol:Milli-Q water (50:50) and (B) methanol:Milli-Q water (90:10) was used; the solvent program was a linear gradient of 100% A to 100% B over 30 minutes with a 30-minute hold, then 100% B to 100% A in 1 minute with a 10-minute hold. The flow rate was 0.8 mL/minute.

Karl Fischer titration indicated approximately 747 ppm water. Elemental analyses for carbon, hydrogen, and oxygen were in agreement with the theoretical values for trimethylolpropane triacrylate. GC indicated one major peak and two impurities with areas of 6.5% and 3.4% relative to the major peak area. HPLC indicated a major peak and five impurities with a combined area of 22.2%. HPLC/MS indicated 10 impurities contributing or corresponding to the impurity peaks identified by HPLC. These impurities included four structurally related acrylates or adducts: trimethylolpropane diacrylate, trimethylolpropane triacrylate acrylic acid adduct, trimethylolpropane triacrylate-trimethylolpropane monoacrylate adduct, and trimethylolpropane triacrylate-trimethylolpropane diacrylate adduct. No substantial amount of 4-methoxyphenol, a stabilizer added to trimethylolpropane triacrylate, was detected. The overall purity of lot 01031AW was estimated to be approximately 80%.

To ensure stability, the bulk chemical was stored at room temperature, protected from light, in amber glass bottles with Teflon®-lined lids. Stability was monitored throughout the studies with GC by systems similar to system A (2-week and 3-month studies) or B (6-month study). No degradation of the bulk chemical was detected.

12-*O*-Tetradecanoylphorbol-13-acetate

12-*O*-Tetradecanoylphorbol-13-acetate was obtained from Sigma Chemical Company (St. Louis, MO) in one lot (48H1178) for use in the 6-month study. Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC). The bulk chemical was stored in its original containers, protected from light, at –20° C or less.

The chemical was identified as 12-*O*-tetradecanoylphorbol-13-acetate by infrared and proton NMR spectroscopy. All spectra were consistent with the structure of 12-*O*-tetradecanoylphorbol-13-acetate.

The purity was determined with HPLC using a Zorbax Rx C_8 column (250 mm × 4.6 mm, 5-µm particle size; DuPont, Wilmington, DE) and ultraviolet detection at 232 nm. A mobile phase of water:acetonitrile (10:90) (isocratic) was used; the flow rate was 1.0 mL/minute. HPLC indicated a major peak, one impurity peak with an area of approximately 0.11% of the total peak area, and two minor impurities with areas less than 0.1% of the total peak area. The overall purity was determined to be greater than 99%.

Acetone

Acetone was obtained in two lots from Honeywell Burdick and Jackson (Muskegon, MI) (lots BK792 and BL631) and in five lots from Spectrum Chemical Manufacturing Corporation **(**Gardena, CA**)** (lots JE342, KP206, LS0051, MI0172, and NE0173). Lots BK792, BL631, and JE342 were used in the 2-week studies, lots KP206 and LS0051 were used in the 3-month studies, and lots MI0172 and NE0173 were used in the 6-month study. Identity and purity analyses of lots BL631 and JE342 and all lots used in the 3- and 6-month studies were conducted by the analytical chemistry laboratory (Midwest Research Institute, Kansas City, MO) and the study laboratory.

The chemical, a clear liquid, was identified as acetone by the analytical chemistry laboratory (lots BL631, JE342, KP206, and LS0051) or the study laboratory (lots MI0172 and NE0173) using infrared spectroscopy. All spectra were consistent with a literature spectrum (*Aldrich*, 1985) or with the structure of acetone.

The purity was analyzed by the analytical chemistry laboratory (lots BL631, JE342, KP206, and LS0051) or the study laboratory (lots MI0172 and NE0173) using GC by systems similar to system C (lots JE342, MI0172, and NE0173) and by system D (lots BL631, KP206, and LS0051). No significant impurities were detected in any lot. The overall purity of each lot was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored in amber glass bottles at room temperature. Stability was monitored with GC by system C. No degradation of the acetone was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared twice (2-week studies) or every 4 weeks by mixing trimethylolpropane triacrylate and acetone to give the required concentration (Table H2). The dose formulations were stored for up to 35 days at room temperature in amber glass bottles with Teflon®-lined lids except dose formulations prepared on September 3 and 6, 1996 (3-month studies), which were stored in amber (rat) or clear (mouse) glass vials with Teflon® septa at –20° C or less. Positive control formulations for the 6-month study were prepared twice by mixing 12-*O*-tetradecanoylphorbol-13-acetate with acetone to provide a concentration of 12.5 µg/mL.

Stability studies of the 6.25 and 100 mg/mL dose formulations for the 2-week studies as well as 50 and 400 µg/mL dose formulations were performed by the study laboratory with GC by systems similar to system B. Stability was confirmed for at least 35 days for dose formulations stored in amber glass bottles with Teflon®-lined lids or septa, with minimal headspace, at temperatures up to 25° C and for 3 hours under animal room conditions, periodically or continually exposed to air and light.

Periodic analyses of the dose formulations of trimethylolpropane triacrylate were conducted by the study laboratory using GC by systems similar to system B. During the 2-week studies, the dose formulations were analyzed once; all five dose formulations for rats and three of five for mice were within 10% of the target concentrations (Table H3). Animal room samples of these dose formulations were also analyzed; four of five animal room samples for rats and all samples for mice were within 10% of the target concentrations. The dose formulations were analyzed at the beginning, midpoint, and end of the 3-month studies; animal room samples of

these dose formulations were also analyzed (Table H4). Of the dose formulations analyzed, 14 of 15 for rats and 15 of 15 for mice were within 10% of the target concentrations, with no value greater than 103% of the target concentration; 12 of 15 of the animal room samples for rats and 14 of 15 for mice were within 10% of the target concentrations. During the 6-month study, the dose formulations were analyzed approximately every 8 or 12 weeks (Table H5). Of the dose formulations analyzed, 14 of 15 were within 10% of the target concentrations; all five animal room samples were within 10% of the target concentrations. In all studies, dose formulations that were not within 10% of the target concentrations were remixed and reanalyzed; all dose formulations analyzed that were administered to rats and mice were within specifications. The positive control formulations were analyzed by the analytical chemistry laboratory using HPLC with a system similar to that described for the positive control purity analysis and were found to be within 10% of the target concentration (data not shown).

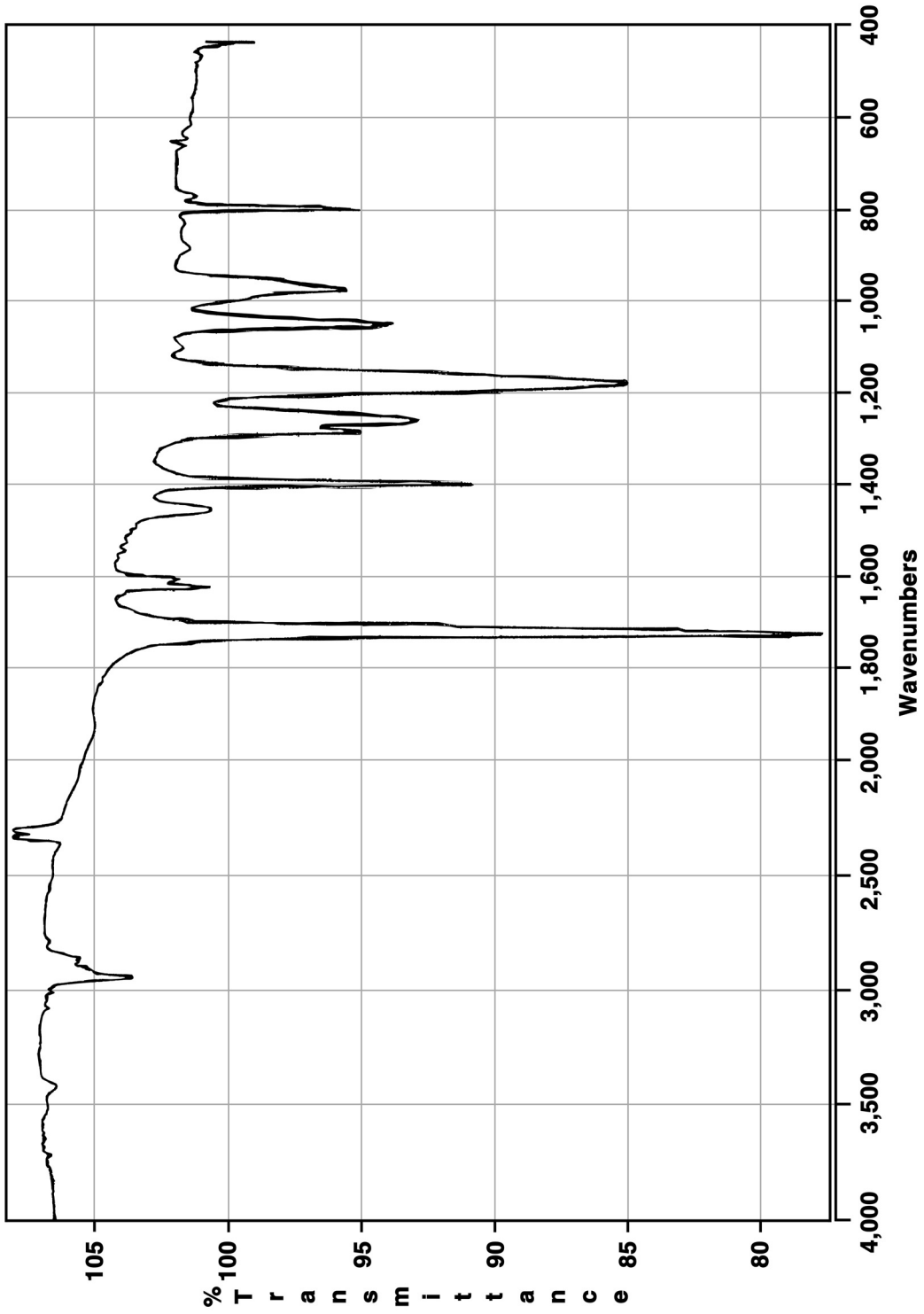

FIGURE H1
Infrared Absorption Spectrum of Trimethylolpropane Triacrylate

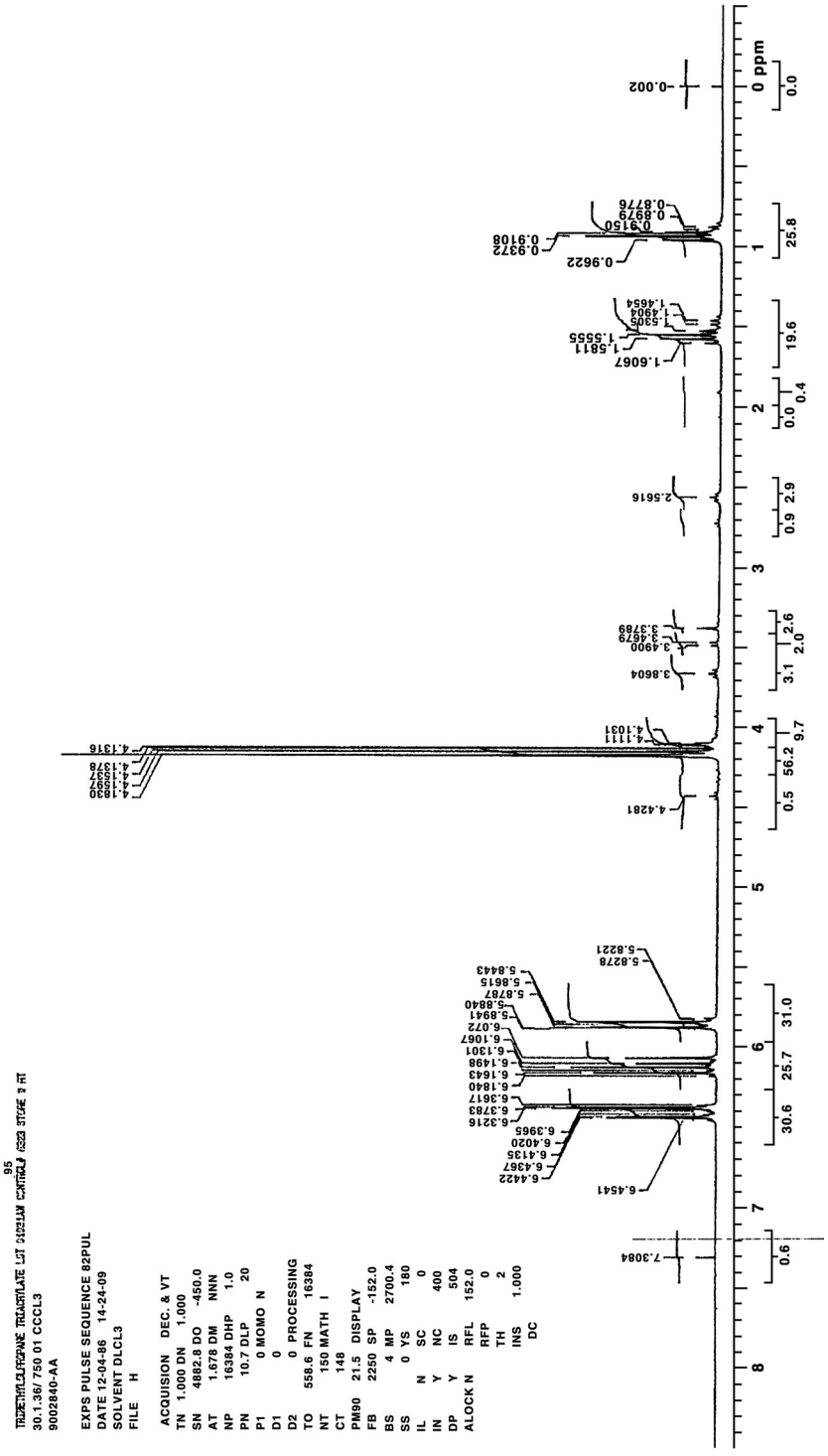

FIGURE H2
Proton Nuclear Magnetic Resonance Spectrum of Trimethylolpropane Triacrylate

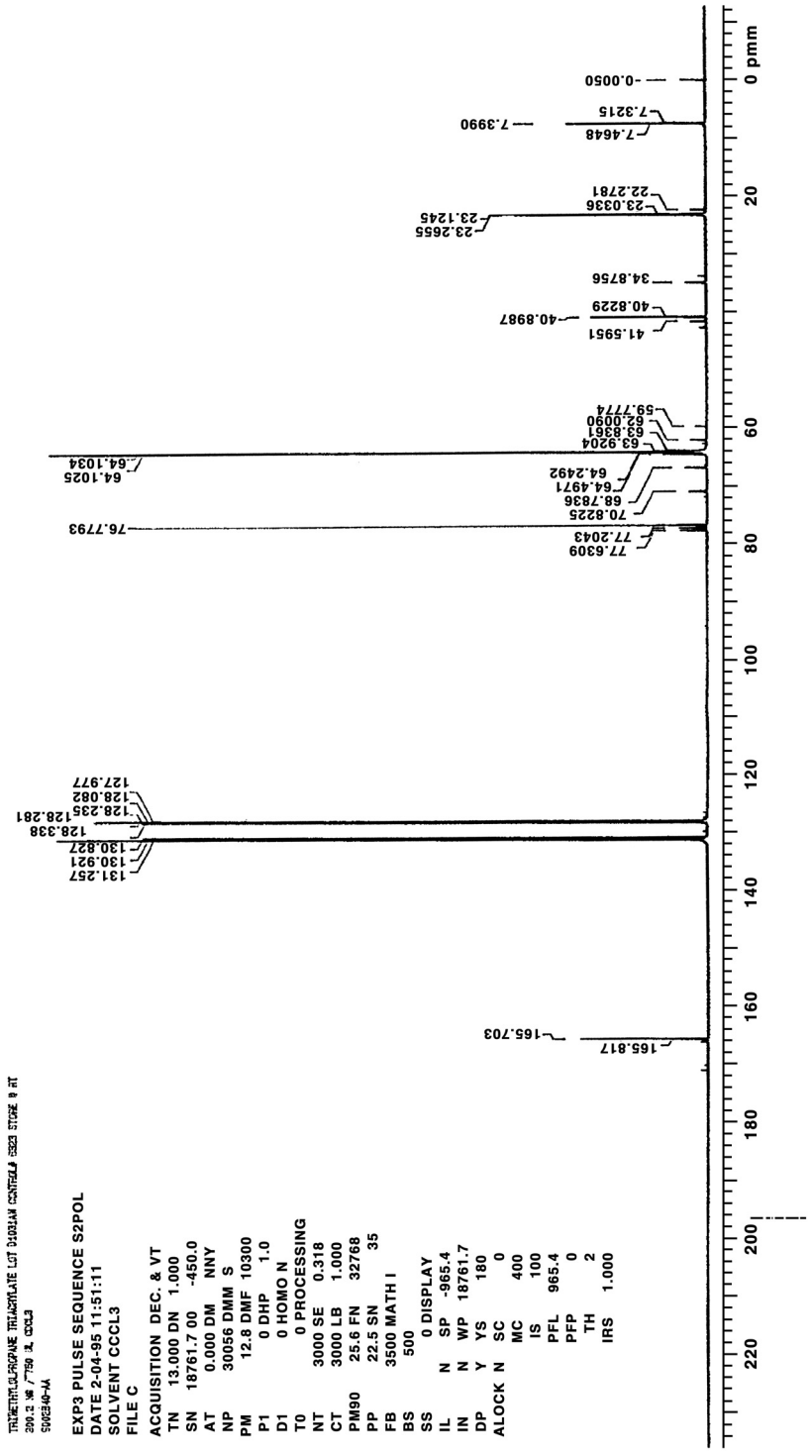

FIGURE H3
^{13}C Nuclear Magnetic Resonance Spectrum of Trimethylolpropane Triacrylate

TABLE H1
Gas Chromatography Systems Used in the Dermal Studies of Trimethylolpropane Triacrylate

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	DB-5, 30 m × 0.32 mm, 0.25-µm film thickness (J&W Scientific, Folsom, CA)	Helium at 5 mL/minute	70° C for 2.5 minutes, then 9° C/minute to 210° C, held 10 minutes
System B			
Flame ionization	RTX-5, 30 m × 0.25 mm, 0.25-µm film thickness (Restek, Bellefonte, PA)	Helium at approximately 3 mL/minute	70° C for 2.5 minutes, then 9° C/minute to 210° C, held 10 minutes
System C			
Flame ionization	20% SP-2401/0.1% Carbowax on 100/120 Supelcoport, 2.4 m × 2 mm	Nitrogen or helium at approximately 30 mL/minute	40° C for 4 minutes, then 10° C/minute to 170° C
System D			
Flame ionization	DB-WAX, 30 m × 0.53 mm, 1-µm film thickness (J&W Scientific)	Helium at 10 mL/minute	80° C for 5 minutes, then 10° C/minute to 220° C, held 4 minutes

TABLE H2
Preparation and Storage of Dose Formulations in the Dermal Studies of Trimethylolpropane Triacrylate

2-Week Studies	3-Month Studies	6-Month Study
Preparation		
Trimethylolpropane triacrylate was manually shaken or sonicated with acetone. Dose formulations were prepared twice.	Same as 2-week studies. Dose formulations were prepared every 4 weeks.	Same as 3-month studies
Trimethylolpropane Triacrylate Lot Number		
01031AW	01031AW	01031AW
Maximum Storage Time		
35 days	35 days	35 days
Storage Conditions		
Stored in amber glass bottles with Teflon®-lined lids at room temperature	Formulations prepared on September 3 and 6, 1996, were stored in amber or clear glass vials with Teflon® septa at –20° C or less; other formulations were stored same as 2-week studies.	Same as 2-week studies
Study Laboratory		
Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)

TABLE H3
Results of Analyses of Dose Formulations Administered to Rats and B6C3F$_1$ Mice in the 2-Week Dermal Studies of Trimethylolpropane Triacrylate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
May 1, 1996	May 2, 1996	25	24.28	–3
		50	49.41	–1
		100	97.87	–2
		200	188.7	–6
		400	392.8	–2
	May 22-24, 1996[b]	25	26.11	+4
		50	52.80	+6
		100	106.0[c]	+6
		200	192.1	–4
		400	457.8	+14
Mice				
May 1, 1996	May 2, 1996	6.25	—[d,e]	—
		12.5	21.72[e]	+74
		25	24.28	–3
		50	49.41	–1
		100	97.87	–2
May 3, 1996	May 4, 1996	6.25	7.198[e,f]	+15
		12.5	7.954[e,f]	–36
May 6, 1996	May 7, 1996	6.25	5.748[f]	–8
		12.5	11.95[f]	–4
	May 22-24, 1996[b]	6.25	6.225	0
		12.5	12.96	+4
		25	25.66	+3
		50	52.04	+4
		100	104.9	+5

[a] Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 25 mg/mL=12.5 mg/kg; 50 mg/mL=25 mg/kg; 100 mg/mL=50 mg/kg; 200 mg/mL=100 mg/kg; 400 mg/mL=200 mg/kg. For mice, dosing volume=2.0 mL/kg; 6.25 mg/mL=12.5 mg/kg; 12.5 mg/mL=25 mg/kg; 25 mg/mL=50 mg/kg; 50 mg/mL=100 mg/kg; 100 mg/mL=200 mg/kg
[b] Animal room samples
[c] Results of quadruplicate analyses
[d] Not quantifiable
[e] Remixed; not used in study
[f] Results of remix

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and B6C3F$_1$ Mice in the 3-Month Dermal Studies of Trimethylolpropane Triacrylate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
September 3, 1996	September 5, 1996	1.5	1.514	+1
		3	3.038	+1
		6	6.033	+1
		12	8.653[b]	–28
		24	24.66	+3
September 6, 1996	September 6, 1996	12	11.94[c]	0
	October 4, 1996[d]	1.5	1.556	+4
		3	3.076	+3
		6	6.207	+3
		12	12.27	+2
		24	24.72	+3
October 28, 1996	October 29, 1996	1.5	1.503	0
		3	3.011	0
		6	5.944	–1
		12	11.84	–1
		24	23.51	–2
	December 2-3, 1996[d]	1.5	1.529	+2
		3	3.205	+7
		6	6.854	+14
		12	13.65	+14
		24	26.59	+11
November 25, 1996	November 25, 1996	1.5	1.514	+1
		3	3.005	0
		6	6.024	0
		12	12.00	0
		24	23.81	–1
	December 31, 1996[d]	1.5	1.493	0
		3	3.019	+1
		6	6.283	+5
		12	11.86	–1
		24	23.88	0

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and B6C3F$_1$ Mice in the 3-Month Dermal Studies of Trimethylolpropane Triacrylate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
September 3, 1996	September 5, 1996	0.375	0.3848	+3
		0.75	0.7620	+2
		1.5	1.514	+1
		3	3.038	+1
		6	6.033	+1
	October 4, 1996[d]	0.375	0.3892	+4
		0.75	0.7772	+4
		1.5	1.567	+4
		3	3.082	+3
		6	6.280	+5
October 28, 1996	October 29, 1996	0.375	0.3629	–3
		0.75	0.7475	0
		1.5	1.503	0
		3	3.011	0
		6	5.944	–1
	December 2-3, 1996[d]	0.375	0.3880	+3
		0.75	0.7769	+4
		1.5	1.548	+3
		3	3.168	+6
		6	6.762	+13
November 25, 1996	November 25, 1996	0.375	0.3780	+1
		0.75	0.7362	–2
		1.5	1.514	+1
		3	3.005	0
		6	6.024	0
	December 31, 1996[d]	0.375	0.3673	–2
		0.75	0.7500	0
		1.5	1.539	+3
		3	3.051	+2
		6	6.264	+4

[a] Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 1.5 mg/mL=0.75 mg/kg; 3 mg/mL=1.5 mg/kg; 6 mg/mL=3 mg/kg; 12 mg/mL=6 mg/kg; 24 mg/mL=12 mg/kg. For mice, dosing volume=2.0 mL/kg; 0.375 mg/mL=0.75 mg/kg; 0.75 mg/mL=1.5 mg/kg; 1.5 mg/mL=3 mg/kg; 3 mg/mL=6 mg/kg; 6 mg/mL=12 mg/kg
[b] Remixed; not used in study
[c] Results of remix
[d] Animal room samples

TABLE H5
Results of Analyses of Dose Formulations Administered to Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Trimethylolpropane Triacrylate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
July 14, 1998	July 15, 1998	0.227	0.2542[b]	+12
		0.455	0.4797	+5
		0.909	0.9867	+9
		1.82	1.930	+6
		3.64	3.676	+1
July 16, 1998	July 16, 1998	0.227	0.2188[c]	–4
	August 20-21, 1998[d]	0.227	0.2252	–1
		0.455	0.4675	+3
		0.909	0.9396	+3
		1.82	1.881	+3
		3.64	3.666	+1
September 9, 1998	September 10-11, 1998	0.227	0.2088	–8
		0.455	0.4224	–7
		0.909	0.8422	–7
		1.82	1.754	–4
		3.64	3.589	–1
December 1, 1998	December 2-3, 1998	0.227	0.2304	+1
		0.455	0.4603	+1
		0.909	0.9200	+1
		1.82	1.887	+4
		3.64	3.741	+3

[a] Results of duplicate analyses. Dosing volume=3.3 mL/kg; 0.227 mg/mL=0.75 mg/kg; 0.455 mg/mL=1.5 mg/kg; 0.909 mg/mL=3 mg/kg; 1.82 mg/mL=6 mg/kg; 3.64 mg/mL=12 mg/kg
[b] Remixed; not used in study
[c] Results of remix
[d] Animal room samples (0.227 mg/mL formulation from July 16, 1998, mix; all others from July 14, 1998, mix).

APPENDIX I
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE I1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE I2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE I3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	13.0 ± 0.26	12.7 – 13.3	7
Crude fat (% by weight)	8.1 ± 0.26	7.6 – 8.4	7
Crude fiber (% by weight)	9.6 ± 0.36	9.2 – 10.0	7
Ash (% by weight)	4.9 ± 0.11	4.7 – 5.0	7
Amino Acids (% of total diet)			
Arginine	0.731 ± 0.050	0.670 – 0.800	8
Cystine	0.224 ± 0.012	0.210 – 0.240	8
Glycine	0.684 ± 0.041	0.620 – 0.740	8
Histidine	0.333 ± 0.018	0.310 – 0.350	8
Isoleucine	0.524 ± 0.046	0.430 – 0.590	8
Leucine	1.061 ± 0.061	0.960 – 1.130	8
Lysine	0.708 ± 0.056	0.620 – 0.790	8
Methionine	0.401 ± 0.035	0.350 – 0.460	8
Phenylalanine	0.598 ± 0.036	0.540 – 0.640	8
Threonine	0.501 ± 0.051	0.430 – 0.590	8
Tryptophan	0.126 ± 0.014	0.110 – 0.150	8
Tyrosine	0.390 ± 0.056	0.280 – 0.460	8
Valine	0.640 ± 0.049	0.550 – 0.690	8
Essential Fatty Acids (% of total diet)			
Linoleic	3.97 ± 0.284	3.59 – 4.54	8
Linolenic	0.30 ± 0.042	0.21 – 0.35	8
Vitamins			
Vitamin A (IU/kg)	6,077 ± 946	4,820 – 7,420	7
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	82.2 ± 14.08	62.2 – 107.0	8
Thiamine (ppm)[b]	7.3 ± 1.25	6.1 – 9.3	7
Riboflavin (ppm)	5.6 ± 1.12	4.20 – 7.70	8
Niacin (ppm)	74.3 ± 5.94	66.4 – 85.8	8
Pantothenic acid (ppm)	22.5 ± 3.96	17.4 – 29.1	8
Pyridoxine (ppm)[b]	9.04 ± 2.37	6.4 – 12.4	8
Folic acid (ppm)	1.64 ± 0.38	1.26 – 2.32	8
Biotin (ppm)	0.333 ± 0.15	0.225 – 0.704	8
Vitamin B_{12} (ppb)	68.7 ± 63.0	18.3 – 174.0	8
Choline (ppm)[b]	3,155 ± 325	2,700 – 3,790	8
Minerals			
Calcium (%)	0.958 ± 0.015	0.935 – 0.979	7
Phosphorus (%)	0.532 ± 0.020	0.505 – 0.563	7
Potassium (%)	0.659 ± 0.022	0.627 – 0.691	8
Chloride (%)	0.357 ± 0.027	0.300 – 0.392	8
Sodium (%)	0.189 ± 0.019	0.160 – 0.212	8
Magnesium (%)	0.199 ± 0.009	0.185 – 0.213	8
Sulfur (%)	0.178 ± 0.021	0.153 – 0.209	8
Iron (ppm)	160 ± 14.7	135 – 177	8
Manganese (ppm)	50.3 ± 4.82	42.1 – 56.0	8
Zinc (ppm)	50.7 ± 6.59	43.3 – 61.1	8
Copper (ppm)	6.29 ± 0.828	5.08 – 7.59	8
Iodine (ppm)	0.461 ± 0.187	0.233 – 0.843	8
Chromium (ppm)	0.542 ± 0.128	0.330 – 0.707	7
Cobalt (ppm)	0.23 ± 0.049	0.20 – 0.30	7

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE I4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.18 ± 0.090	0.10 – 0.33	7
Cadmium (ppm)	0.04 ± 0.004	0.04 – 0.05	7
Lead (ppm)	0.08 ± 0.008	0.07 – 0.09	7
Mercury (ppm)	<0.02		7
Selenium (ppm)	0.18 ± 0.024	0.15 – 0.23	7
Aflatoxins (ppb)	<5.00		7
Nitrate nitrogen (ppm)[c]	11.2 ± 2.75	9.04 – 16.8	7
Nitrite nitrogen (ppm)[c]	<0.61		7
BHA (ppm)[d]	<1.0		7
BHT (ppm)[d]	<1.0		7
Aerobic plate count (CFU/g)	<10		7
Coliform (MPN/g)	0		7
Escherichia coli (MPN/g)	<10		7
Salmonella (MPN/g)	Negative		7
Total nitrosoamines (ppb)[e]	5.8 ± 1.89	3.2 – 8.8	7
N-Nitrosodimethylamine (ppb)[e]	2.5 ± 1.31	1.2 – 5.1	7
N-Nitrosopyrrolidine (ppb)[e]	3.3 ± 1.3	1.9 – 5.6	7
Pesticides (ppm)			
α-BHC	<0.01		7
β-BHC	<0.02		7
γ-BHC	<0.01		7
δ-BHC	<0.01		7
Heptachlor	<0.01		7
Aldrin	<0.01		7
Heptachlor epoxide	<0.01		7
DDE	<0.01		7
DDD	<0.01		7
DDT	<0.01		7
HCB	<0.01		7
Mirex	<0.01		7
Methoxychlor	<0.05		7
Dieldrin	<0.01		7
Endrin	<0.01		7
Telodrin	<0.01		7
Chlordane	<0.05		7
Toxaphene	<0.10		7
Estimated PCBs	<0.20		7

TABLE I4
Contaminant Levels in NTP-2000 Rat and Mouse Ration

	Mean ± Standard Deviation	Range	Number of Samples
Pesticides (ppm) (continued)			
Ronnel	<0.01		7
Ethion	<0.02		7
Trithion	<0.05		7
Diazinon	<0.10		7
Methyl chlorpyrifos	0.075 ± 0.081	0.020 – 0.253	7
Methyl parathion	<0.02		7
Ethyl parathion	<0.02		7
Malathion	0.121 ± 0.118	0.020 – 0.311	7
Endosulfan I	<0.01		7
Endosulfan II	<0.01		7
Endosulfan sulfate	<0.03		7

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All values were corrected for percent recovery.

APPENDIX J
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from randomly selected rats and mice during the 3- and 6-month studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to MA BioServices, Inc. (Rockville, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
3-Month Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	4 weeks, study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	4 weeks, study termination
Sendai	4 weeks, study termination
Immunofluorescence Assay	
Helicobacter hepaticus	Study termination
PVM	Study termination
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	4 weeks, study termination
KRV (Kilham rat virus)	4 weeks, study termination

Method and Test	Time of Analysis
MICE	
3-Month Study	
ELISA	
Ectromelia virus	4 weeks, study termination
EDIM (epizootic diarrhea of infant mice)	4 weeks, study termination
GDVII (mouse encephalomyelitis virus)	4 weeks, study termination
LCM (lymphocytic choriomeningitis virus)	4 weeks, study termination
Mouse adenoma virus	4 weeks, study termination
MHV (mouse hepatitis virus)	4 weeks, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	4 weeks, study termination
Reovirus 3	4 weeks, study termination
Sendai	4 weeks, study termination
Immunofluorescence Assay	
H. hepaticus	Study termination
LCM	4 weeks
MCMV (mouse cytomegalovirus)	Study termination
Hemagglutination Inhibition	
K (papovavirus)	4 weeks, study termination
MVM (minute virus of mice)	4 weeks, study termination
Polyoma virus	4 weeks, study termination
6-Month Study	
ELISA	
Ectromelia virus	4 weeks, study termination
EDIM	4 weeks, study termination
GDVII	4 weeks, study termination
LCM	4 weeks, study termination
Mouse adenoma virus-FL	4 weeks, study termination
MHV	4 weeks, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	4 weeks, study termination
Reovirus 3	4 weeks, study termination
Sendai	4 weeks, study termination
Immunofluorescence Assay	
MCMV	Study termination
Parvovirus	4 weeks, study termination

RESULTS

One female rat in the 3-month study had a positive titer for *M. arthritidis*. Further evaluation of the sample by immunoblot and Western blot procedures indicated that the positive titer may have been due to cross reaction with antibodies of nonpathogenic *Mycoplasma* or other agents. Only a single sample was positive and there were no clinical findings or histopathologic changes of *M. arthritidis* infection in the animal with the positive titer. Accordingly, the *M. arthritidis*-positive titer was considered a false positive. For the 3- and 6-month studies in mice, all serology tests were negative.

APPENDIX K
CONTACT HYPERSENSITIVITY STUDIES

CONTACT HYPERSENSITIVITY STUDIES

INTRODUCTION

Studies were conducted with female BALB/c mice to evaluate the potential for trimethylolpropane triacrylate to induce contact hypersensitization. A primary irritancy study of trimethylolpropane triacrylate was performed to screen for toxicity and determine the maximal nonirritating and minimal irritating concentrations for a mouse ear swelling test and a local lymph node assay, two assessments of the dermal sensitizing potential of the compound. The studies were performed by the Medical College of Virginia Immunotoxicology Laboratory (Virginia Commonwealth University, Richmond, VA).

MATERIALS AND METHODS

Trimethylolpropane triacrylate (lot 01031AW) was obtained from Aldrich Chemical Company (Milwaukee, WI). Acetone was used as the vehicle. Analyses of the bulk chemicals are described in Appendix H. Dose formulations were prepared daily by mixing trimethylolpropane triacrylate in acetone.

Female BALB/c mice were obtained from the National Cancer Institute (Bethesda, MD). The mice were approximately 6 to 7 weeks of age at receipt and were quarantined for at least 6 days; serology tests indicated that the animals were free of viral (mouse hepatitis and Sendai) and bacterial (*Mycoplasma*) contamination. Mice received certified NIH-07 rodent feed and tap water in water bottles *ad libitum*. Mice were housed no more than five per cage in plastic shoe-box type cages with sawdust bedding; the cages were cleaned and sanitized twice per week.

For the irritancy study (Figure K1), groups of four mice received 50 µL dermal applications of 0% (vehicle controls), 0.025%, 0.05%, 0.075%, 0.1%, 0.25%, or 0.5% (w/v) trimethylolpropane triacrylate in acetone; 12.5 µL were applied to each side of each ear. Doses were administered by pipette once per day for 4 consecutive days. An additional group of four animals was maintained as untreated (naive) controls. Prior to application of the first dose and 24 ± 2 hours after the last dose, the thickness of each ear was measured at two sites with a modified micrometer (Mitutoyo America Corp., Aurora, IL). Ear swelling (mean thickness after dosing/mean thickness predosing) was calculated as a percentage for each ear.

For the mouse ear swelling test (Figure K2), groups of eight mice were sensitized with 50 µL dermal applications of 0% (two control groups), 0.01%, 0.05%, or 0.1% (w/v) trimethylolpropane triacrylate in acetone to the shaved dorsal lumbar area. Doses were administered by pipette once per day for 3 consecutive days. The animals were restrained after dosing to allow the vehicle to begin to volatilize. The mice were not dosed on days 4 through 7. On day 8, the thickness of the right ear of each animal was measured prior to dosing as described for the irritancy study. Challenge doses (25.0 µL total volume) were applied to the dorsal and ventral surfaces of the right ear pinna, divided between the two sides. The two control groups sensitized with acetone received challenges of acetone (vehicle controls) or 0.25% trimethylolpropane triacrylate (background controls). The three groups sensitized with trimethylolpropane triacrylate received a 0.25% trimethylolpropane triacrylate challenge dose. The right ears were remeasured 24 and 48 hours after the challenge doses were applied, and ear swelling was calculated as a percentage for each mouse at each time point.

For the local lymph node assay (Figure K3), groups of six mice were sensitized with 50 µL dermal applications of 0% (vehicle controls), 0.05%, 0.1%, or 0.25% (w/v) trimethylolpropane triacrylate in acetone. The doses (12.5 µL applied to each side of each ear) were applied by pipette once per day for 3 consecutive days. The mice were not dosed on day 4. On day 5, 0.2 mL (20 µCi) of [^{3}H]-thymidine was intravenously injected (tail vein). The animals were killed 5 hours after the injection, and the left and right draining (superficial cervical) lymph nodes were excised and placed in cold phosphate-buffered saline. All lymph nodes were dissociated by grinding between the

frosted ends of two microscope slides. The cells were washed twice in phosphate-buffered saline and then resuspended in 3 mL 5% trichloroacetic acid in distilled water. After 18 to 60 hours at approximately 4° C, the cells were resuspended in trichloroacetic acid, transferred into 5 mL scintillation cocktail, and counted on a beta counter for 5 minutes.

The data were analyzed for homogeneity with Bartlett's chi-square test (Bartlett, 1937). Homogeneous data were tested for significance with a one-way analysis of variance (Kruskal and Wallis, 1952) followed by Dunnett's multiple-range *t*-test (Dunnett, 1955) if the analysis of variance indicated a significant main effect. For nonhomogeneous data, a nonparametric analysis of variance, Wilson's test (Wilson, 1956), and the Wilcoxon rank sum test (Gross and Clark, 1975) were used to compare treatment groups with the controls.

Results and Discussion

There were no deaths, body weight changes, or clinical findings related to trimethylolpropane triacrylate treatment in dosed mice. Results of the irritancy study indicated that the maximal nonirritating and minimal irritating doses were 0.1% and 0.25% trimethylolpropane triacrylate, respectively (Figure K4).

In the mouse ear swelling test, no significant differences in the percentage of ear swelling were observed between trimethylolpropane triacrylate-sensitized and -challenged mice and the background controls at 24 or 48 hours after dosing (Figure K5). The local lymph node assay indicated no significant increase in lymph node cell proliferation in mice administered trimethylolpropane triacrylate compared to that in the vehicle controls (Figures K4 to K6).

Testing for sensitizing potential using the mouse ear swelling test and local lymph node assay failed to indicate trimethylolpropane triacrylate as a potential contact sensitizer at the concentrations tested.

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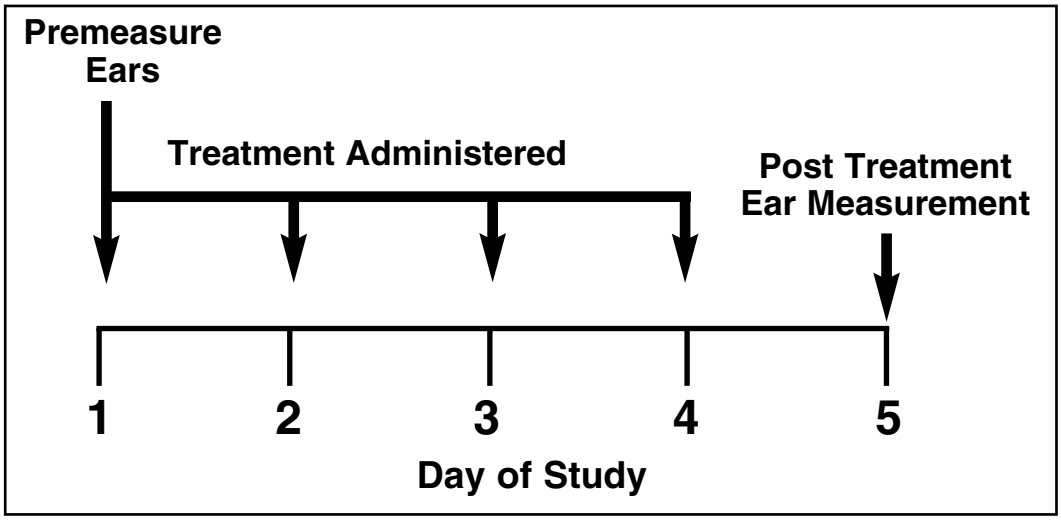

FIGURE K1
Major Events of the Primary Irritancy Study

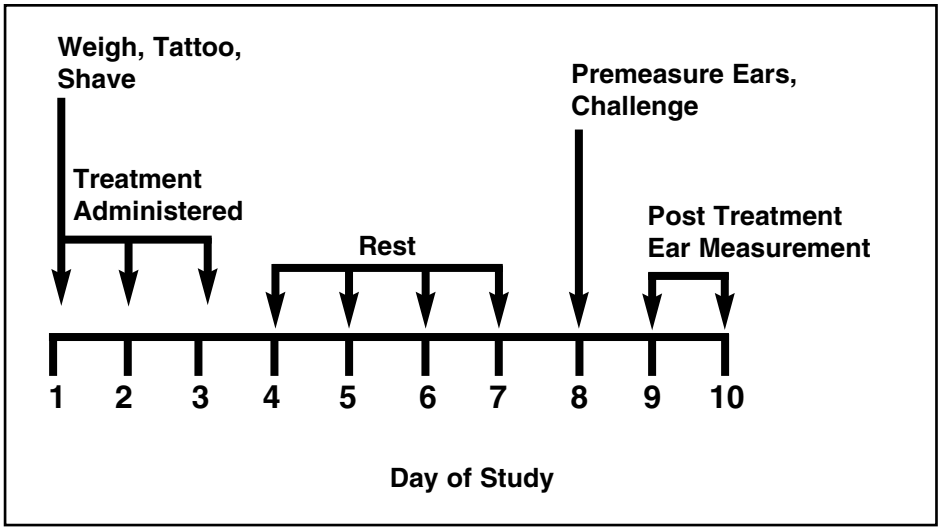

FIGURE K2
Major Events of the Mouse Ear Swelling Test

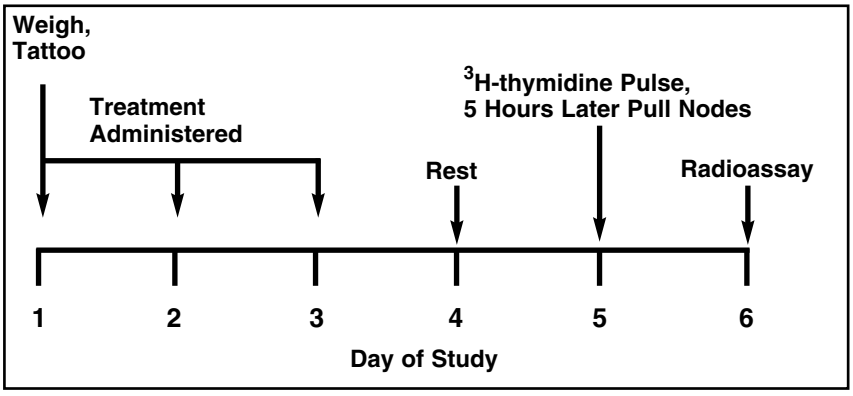

FIGURE K3
Major Events of the Local Lymph Node Assay

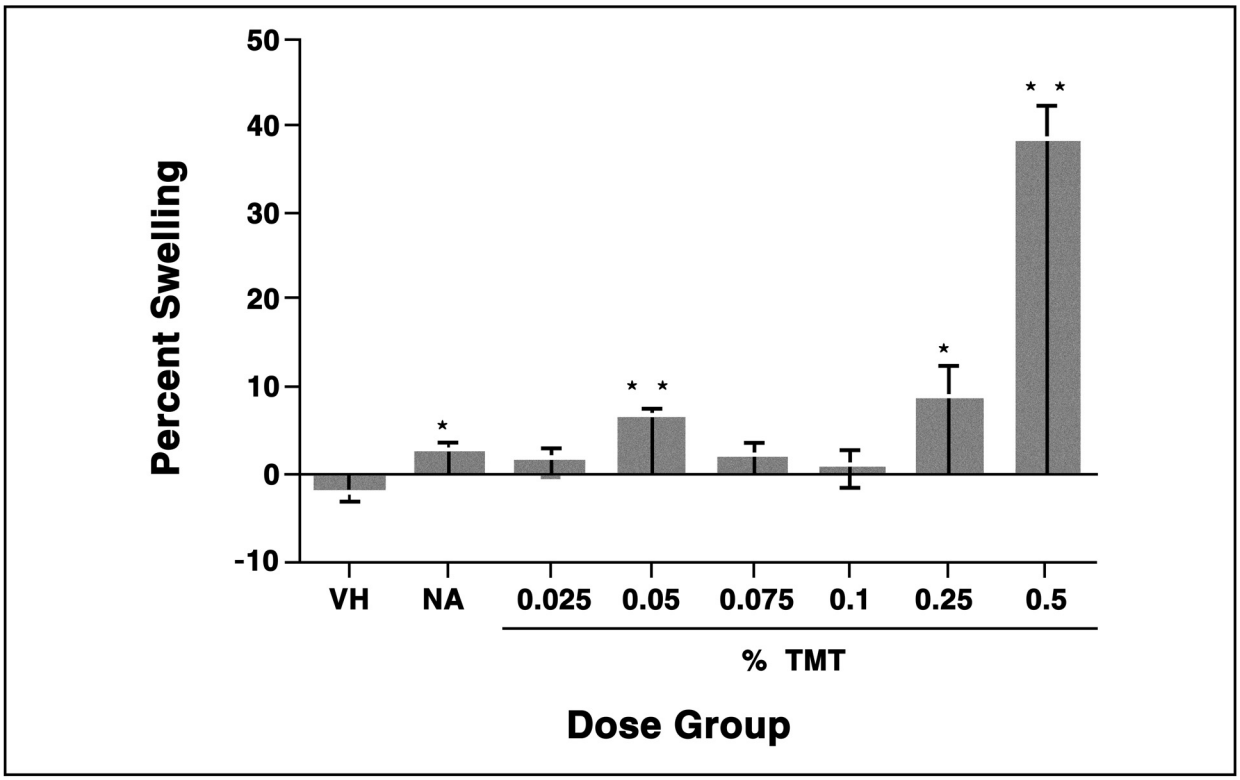

FIGURE K4
Primary Irritancy Response to Trimethylopropane Triacrylate Mixture in Female BALB/c Mice
* Statistically significant (P<0.05) compared to vehicle control
** P<0.01
Means for each group are shown, with bars representing the standard error.
VH=vehicle controls, NA=untreated (naive) controls,
TMT=trimethylolpropane triacrylate mixture

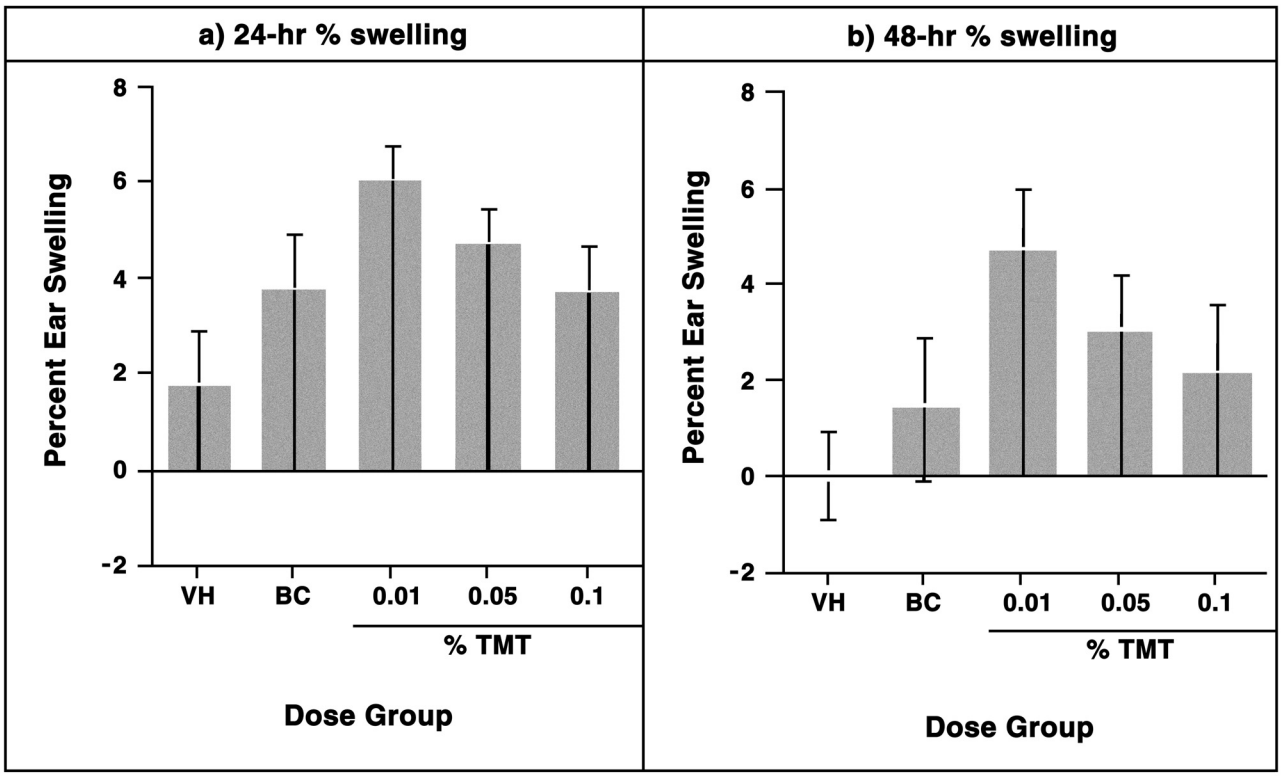

Figure K5
Contact Hypersensitivity Response to Trimethylolpropane Triacrylate Mixture in Female BALB/c Mice (Mouse Ear Swelling Test)
Means for each group are shown, with bars representing the standard error.
VH=vehicle controls, BC=background controls,
TMT=trimethylolpropane triacrylate mixture

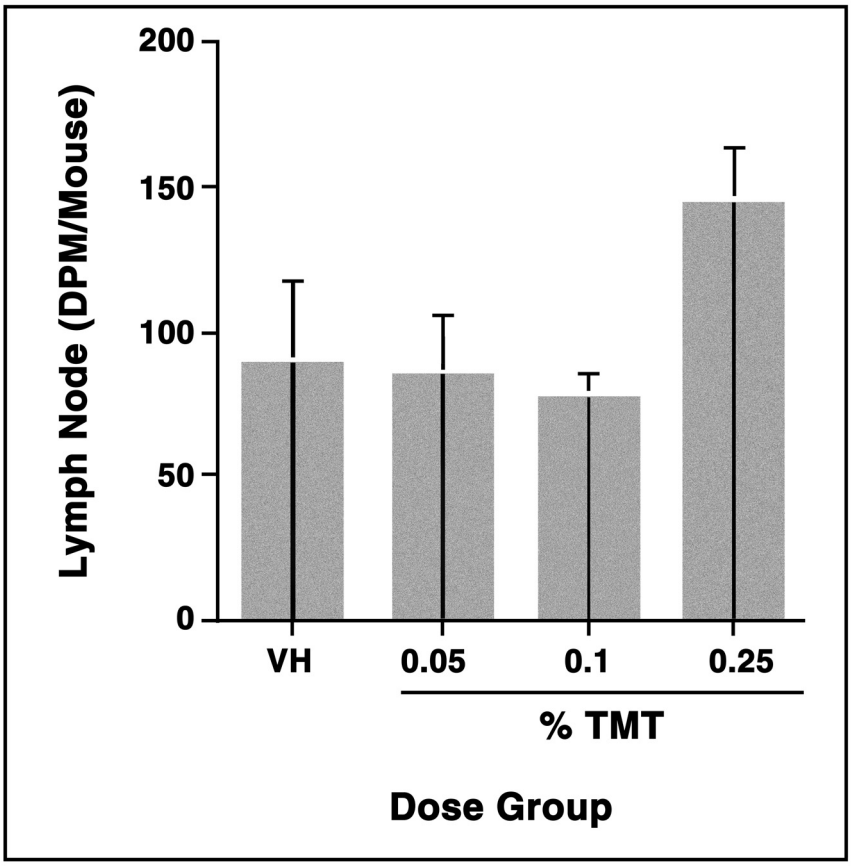

Figure K6
Contact Hypersensitivity Response to Trimethylolpropane Triacrylate Mixture in Female BABL/c Mice (Local Lymph Node Assay)
Means for each group are shown, with bars representing the standard error.
DPM=disintegrations per minute, VH=vehicle controls,
TMT=trimethylolpropane triacrylate mixture

APPENDIX L
ABSORPTION, DISTRIBUTION, AND EXCRETION STUDIES

ABSORPTION, DISTRIBUTION, AND EXCRETION STUDIES

INTRODUCTION

Studies were conducted in adult male F344/N rats and B6C3F$_1$ mice to determine the absorption, distribution, and excretion of trimethylolpropane triacrylate following intravenous bolus injection or dermal application. These studies were conducted by Research Triangle Institute (Research Triangle Park, NC).

MATERIALS AND METHODS

Technical-grade unlabeled trimethylolpropane triacrylate was obtained from Aldrich Chemical Company (Milwaukee, WI) in two lots (03115TV and 03914PV). Identity and purity analyses were conducted by the study laboratory. Proton- and ^{13}C-nuclear magnetic resonance and mass spectra were consistent with the structure. Reverse phase high-performance liquid chromatography (HPLC) indicated the presence of several ultraviolet-absorbing impurities that were not identified.

[^{14}C]-Trimethylolpropane triacrylate (7.5 mCi, lot 91-350-35-02 and 5 mCi, lot R90-306-69-34) was obtained from Chemsyn Science Laboratories (Lenexa, KS). The chemical's identity was confirmed by coelution of [^{14}C]-trimethylolpropane triacrylate with unlabeled trimethylolpropane triacrylate by reverse phase HPLC. The radiochemical purity of [^{14}C]-trimethylolpropane triacrylate was determined to be less than 70%. After development and application of a purification method, the average radiochemical purity of [^{14}C]-trimethylolpropane triacrylate was determined to be 98.8%.

Young adult male F344/N rats (240-306 g) and adult B6C3F$_1$ mice (25-35 g) were obtained from Charles River Laboratories (Raleigh, NC, and Kingston, NY). Animals were coded by ear tags and quarantined for approximately 1 week. Purina Rodent Chow No. 5002 and tap water were available *ad libitum*. Prior to the experiments, rats and mice were housed up to a maximum of four animals per cage in polycarbonate cages. During the excretion studies, animals were housed individually in glass metabolism chambers that allowed for separate collection of urine, feces, and exhaled CO_2 beginning at least 1 day before the studies.

Rats received a single intravenous injection of 9.4 mg [^{14}C]-trimethylolpropane triacrylate/kg body weight or a single dermal dose of 1.7, 15.2, or 130 mg/kg (13.3, 7.2, 10.5, and 10.5 µCi, respectively). In a dermal-tape stripping experiment in rats, successive strips of the washed application site skin were analyzed following a single 124 mg/kg dose of [^{14}C]-trimethylolpropane triacrylate (3.8 µCi). In a preexposure study, rats were given a dermal dose of 151 mg/kg unlabeled trimethylolpropane triacrylate followed 24 hours later by a single dermal dose of 151 mg/kg [^{14}C]-trimethylolpropane triacrylate (8.9 µCi). Mice received a single dermal dose of 1.2 mg/kg [^{14}C]-trimethylolpropane triacrylate (2.1 µCi). In ancillary tolerance studies, rats were administered single intravenous doses of 0.1, 1, or 10 mg/kg or single dermal doses of 16.2, 34.5, 63.4, 91, or 124 mg/kg unlabeled trimethylolpropane triacrylate.

Rats in the intravenous bolus study were anesthetized with an intramuscular or intraperitoneal dose of 60 mg/kg ketamine:xylazine (7:1) or an intraperitoneal dose of 80 to 100 mg/kg ketamine:xylazine:acepromazine (10:1:1) and then implanted with indwelling jugular cannulae to facilitate the collection of blood samples. The design of the cannula was similar to that of Harms and Ojeda (1974) as modified by McKenna and Bieri (1984). The rats were allowed to recover for at least 1 day before dosing. At the time of dosing, the rats were restrained and the intravenous dose was administered as a bolus injection into a lateral tail vein.

Animals in dermal dosing experiments were sedated with an intramuscular dose of 60 mg/kg ketamine:xylazine (7:1) approximately 24 hours before dermal doses were applied, and the fur on the back of each animal was

clipped. The clipped area was wiped with acetone, dried, and examined; animals with broken skin in the clipped area were excluded from the study. The doses were applied to 1 square inch (rats) or 0.5 square inch (mice) previously outlined with a permanent, felt-tip marker. Before dosing, a self-adhering protective foam appliance with a center-cut window was glued onto the back of each rat with Hollister® medical adhesive (Hollister, Inc., Libertyville, IL). Doses were administered with either a ball-tipped gavage needle and glass syringe equipped with a Teflon®-tipped plunger or a silylated glass wiretrol (Drummond Scientific Co., Broomall, PA) to the square inch of skin exposed through the window in the foam appliance. After dosing was complete, a cloth cover was attached to the foam appliance, and a protective metal cover was secured over the appliance with either Elastoplast® or Coban® adhesive bandage (3M Medical-Surgical Division, St. Paul, MN).

After dosing the mice, a halved tissue capsule (Fisher HistoPrep; Fisher Scientific Company, Pittsburgh, PA), the latticed top of which was covered with 50/50 polyester/cotton sheeting, was glued over the dosing site with Dura Quick Gel super glue (Loctite Corp., Rocky Hill, CT). Doses were administered to the shaved skin of mice with a 50 µL wiretrol.

Radiolabeled dose formulations were prepared with purified [^{14}C]-trimethylolpropane triacrylate, which had been concentrated from HPLC fractions. Individual doses of [^{14}C]-trimethylolpropane triacrylate reconstituted in the appropriate solvent were prepared the day before dosing. The intravenous injection dose was formulated in a mixture of absolute ethanol, Emulphor®, and phosphate-buffered saline by adding appropriate quantities of labeled and nonlabeled trimethylolpropane triacrylate to yield a final specific activity of 5.73 µCi/mg trimethylolpropane triacrylate. All dermal doses were prepared by dissolving appropriate quantities of labeled and/or nonlabeled trimethylolpropane triacrylate in acetone. (Final specific activities of the radiolabeled doses for the 1.2 mg/kg mouse and 1.7, 15.2, 124 (tape stripping), 130, and 151 (preexposure) rat studies were 54, 7.2, 2.6, 0.275, and 0.229 µCi trimethylolpropane triacrylate, respectively.)

For the definitive dermal studies in male rats, groups of four or five animals were administered single dermal applications of 1.7, 15.2, 130, or 151 mg [^{14}C]-trimethylolpropane triacrylate/kg body weight. Animals in the 151 mg/kg group had been preexposed to a single dermal dose of unlabeled trimethylolpropane triacrylate at the same concentration approximately 24 hours before the radiolabeled dose. Urine and feces were collected separately into round-bottom flasks cooled with dry ice. Exhaled $^{14}CO_2$ was collected by passing air from the metabolism cages through two traps containing room temperature 1 N sodium hydroxide. To measure cumulative excretion of radiolabel, collection flasks and traps were changed at 8, 24, 48, and 72 hours postdosing. At 72 hours postdosing, the cages were rinsed, and the dosing appliances were removed from the anesthetized animals. The dosing site skin was washed and collected for analysis of acetone-extractable and nonextractable radiolabel. Skin samples from the 1.7 mg/kg group were not extracted. Blood was withdrawn from the animals into a heparinized syringe by cardiac puncture. The animals were sacrificed by intracardiac injection of Euthanasia-6® (Sparhawk Veterinary Laboratories, Inc., Lenexa, KS) or by carbon dioxide asphyxiation. Bladder urine was removed from each animal and added to the final urine collection. Selected tissues were removed from the carcasses and the blood, urine, and tissues were stored at –20° C for subsequent analysis of total radiolabel.

Aliquots of urine, cage rinse, skin wash, and breath trap contents were mixed with scintillation cocktail and directly assayed for ^{14}C content by liquid scintillation spectrometry (LSS); aliquots of blood, feces, and tissues were digested in Soluene 350® (Packard Instrument Company, Meriden, CT), neutralized, decolorized with perchloric acid and hydrogen peroxide, and assayed for total radiolabel. Skin samples and the residual carcass were digested in 2 N ethanolic sodium hydroxide prior to analysis for radiolabel. The dosing appliances were cut into eight pieces, added to scintillation vials containing 2 mL methanol, and analyzed by LSS. Appropriate background samples containing the same combination of reagents as the corresponding biological samples were prepared for all sample types.

A group of five male mice was administered a single dermal dose of 1.2 mg [^{14}C]-trimethylolpropane triacrylate/kg. Urine, feces, breath, cage rinse, skin wash, appliance, and tissue samples were collected, digested,

and analyzed as in the definitive dermal studies in rats, except the skin samples were not extracted with acetone prior to digestion.

In the dermal tape stripping experiment, groups of four male rats were given either 0.5 or 72 hours exposure to a single dermal application of 124 mg [^{14}C]-trimethylolpropane triacrylate/kg followed by 15 strippings of residual radiolabel from the washed dosing site using 2.5 inch × 2.5 inch pieces of Scotch tape (3M®, St. Paul, MN). The tape strips and an acetone extract of the dosing site skin were analyzed for radiolabel by LSS and the presence of trimethylolpropane triacrylate by HPLC, respectively.

In the intravenous injection study, a group of five male rats was given a single bolus dose of 9.4 mg [^{14}C]-trimethylolpropane triacrylate/kg. Serial blood collections were taken at 0.08, 0.5, 1, 3, 6, 24, and 48 hours from the indwelling cannula and at 72 hours by cardiac puncture to analyze for blood concentrations of radiolabel. Urine, feces, cage rinse, and exhaled $^{14}CO_2$ were collected and analyzed for cumulative excretion of radiolabel as in the definitive dermal rat studies except that collections were made at 3, 6, 24, 48, and 72 hours postdosing. Tissue samples were collected at 72 hours postdosing and digested and analyzed for radiolabel as described for the definitive dermal studies in rats.

Kidney samples from the 15.2 mg/kg (dermal) and 9.4 mg/kg (intravenous injection) groups were analyzed to determine the extent of covalent binding of radiolabeled trimethylolpropane triacrylate to kidney protein using the methods of Jollow *et al.* (1973). Homogenized samples were precipitated four times with 0.6 M trichloroacetic acid, and each supernatant was assayed for radiolabel by LSS. The final pellet was extracted four times with methanol:water (80:20), and each extract was assayed for radiolabel by LSS. The final extracted pellet was assayed for protein concentration and radiolabel.

Results and Discussion

Combined results of the definitive and ancillary dermal tolerance studies (data not shown) revealed irritation of the skin (erythema and edema) at all dermal dose levels with the lowest doses (1.5 mg/kg in rats and 1.2 mg/kg in mice) exhibiting only slight irritation.

In rats, 18.7% of a single dermal dose of 130 mg [^{14}C]-trimethylolpropane triacrylate/kg was absorbed and an average of 76% of the administered radiolabel was recovered unabsorbed from the appliance and dose site skin 72 hours after dosing (Table L1). In animals administered a 24-hour preexposure dose of nonradiolabeled trimethylolpropane triacrylate, 25% of the subsequent 151 mg/kg radiolabeled dose was absorbed and 65% was recovered unabsorbed from the dose site 72 hours after dosing. These results indicate that preexposed rats absorbed 1.33 times as much [^{14}C]-trimethylolpropane triacrylate as animals that were not preexposed. Because the site of application appeared irritated after a single application of trimethylolpropane triacrylate, chronic exposure may make the skin more permeable to subsequent trimethylolpropane triacrylate exposures. Absorption of dermally administered trimethylolpropane triacrylate was inversely related to dose; a total of 18.7% of the 130 mg/kg dose was absorbed, while 32.7% of the 15.2 mg/kg and 55.1% of the 1.7 mg/kg dose were absorbed. In terms of mass, approximately five times more was trimethylolpropane triacrylate was absorbed as the dose level increased by one order of magnitude. Most of the radiolabel remaining in the animals 72 hours after dermal application was associated with the skin at the dose site. Nonabsorbed test chemical was removed from this skin by thorough washing with soapy water at the end of the dermal study. Acetone extracted 8%, 10%, and 9%, respectively, of the dose from the site of application of rats administered single doses of 15.2, 130 or 151 mg/kg (preexposed). HPLC analysis of the acetone extracts showed that trimethylolpropane triacrylate was the major constituent removed from the skin by acetone 72 hours after exposure (Figure L1).

An average of less than 5% of the dose was recovered in the excreta of rats 72 hours after dermal application of 130 mg/kg, compared to an average of approximately 19% of the 15.2 g/kg dose and 45% of the 1.7 mg/kg dose (Table L2). Very little radioactivity was associated with most of the tissues 72 hours after exposure (Table L3); however, for each of the studies, the kidney had elevated tissue:blood ratios.

In mice, a total of 75% of the dermally applied [^{14}C]-trimethylolpropane triacrylate was absorbed 72 hours after a single dose of 1.2 mg/kg, approximately 1.4 times as much as was absorbed by rats administered a similar dose (Table L4). This result was not surprising because mouse skin is generally considered to be more permeable than rat skin. A much larger percentage of the applied radioactivity remained at the site of application in mice than in rats (31% in mice, 9% in rats). The collected tissues and residual carcass contained less than 2% of the administered dose. Approximately 21% of the dose remained unabsorbed after 72 hours, yet the dose site showed very little skin irritation. Approximately 42% of the dose was excreted by mice in the urine, feces, and exhaled CO_2 by 72 hours after dosing (Table L5), an amount similar to that excreted by rats treated with 1.7 mg/kg. Similar to the studies in rats, very little radiolabel was associated with most of the tissues 72 hours after dosing; the skin, however, did have an elevated tissue:blood ratio (Table L6).

The tape stripping experiment was conducted in rats to further determine whether trimethylolpropane triacrylate is stable on the skin and absorbed as the parent compound. After a single 124 mg/kg dermal application of [^{14}C]-trimethylolpropane triacrylate and wash of the site of application either 30 minutes or 72 hours postdosing, the site was repeatedly stripped with Scotch® tape. The quantity of radiolabel removed by the tape decreased as the tape strip number increased (Figure L2). On average, 1.3% of the radiolabeled dose was removed by tape stripping after a 30-minute exposure and 1.6% of the dose was removed after a 72-hour exposure.

Following 72 hours of exposure, acetone extracts of the stripped, sliced skin were prepared and analyzed by HPLC; the major peak, accounting for approximately 73% of the radiolabel, was associated with trimethylolpropane triacrylate (Figure L3). Two more peaks accounted for 14% and 10% of the radiolabel. Parent trimethylolpropane triacrylate was therefore the major xenobiotic in the skin at the dosing site and most likely the major xenobiotic available to the systemic circulation throughout the studies.

Ancillary tolerance studies indicated that single intravenous injections of 0.1, 1, and 10 mg trimethylolpropane triacrylate/kg into the lateral tail vein of rats were all adequately tolerated (data not shown). During the 72 hours following a bolus dose of 9.4 mg/kg [^{14}C]-trimethylolpropane triacrylate/kg to rats in the definitive intravenous study, a total of 77.4% of the radiolabel was excreted in the urine, feces, and exhaled CO_2 (Table L7). Urine and exhaled CO_2 accounted for the largest percentages (approximately 48% and 20%, respectively). Preliminary stability studies indicated that [^{14}C]-trimethylolpropane triacrylate was chemically unstable in whole blood (data not shown). Accordingly, total radiolabel (but not parent trimethylolpropane triacrylate) was reliably measured in blood, and only slight changes in radiolabel concentrations were observed in blood after 1 hour (Table L8). Among the tissues collected 72 hours after dosing, the highest radiolabel concentration was in the blood (Table L9). The average total recovery of radiolabel was 90% during the 72 hours after the intravenous dose (Table L10).

Elevated kidney:blood ratios of radiolabel (approximately 3.3-11.1) were noted in rats after dermal exposures to [^{14}C]-trimethylolpropane triacrylate (Table L3). However, urinary excretion of radiolabel during the interval from 48 to 72 hours after dosing was minimal (approximately 1%-7%; Table L2). The kidney:blood ratio of radiolabel after intravenous bolus administration was not elevated (0.19; Table L9).

To determine whether the elevated kidney:blood ratios following dermal doses were due to covalent binding of radiolabeled compounds to tissue macromolecules, the binding of ^{14}C to kidney samples from dermal and intravenous studies in rats was analyzed. As shown in Table L11, the nature of binding varied with the route of administration. Approximately 94% of the radiolabel was recovered in the trichloroacetic acid supernatants and approximately 1% in the methanol:water extracts of the kidney homogenates prepared from animals that had been exposed dermally. This result indicates that the high kidney:blood ratios in dermally dosed rats were not due to covalent binding of radiolabeled compounds to kidney proteins. In contrast, the fact that 60% of the radiolabel remained in the trichloroacetic acid-precipitated pellets prepared from the intravenous bolus study indicates that systemically available trimethylolpropane triacrylate results in covalent binding to macromolecules associated with the kidney. Because trimethylolpropane triacrylate is reactive in blood and remains in circulation at substantial

levels (approximately 14 µg-Eq/g blood 72 hours after a 9.4 mg/kg intravenous bolus dose; Table L8), the elevated kidney:blood ratio seen after dermal exposure may be due to urine in the making at the time of necropsy.

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TABLE L1
Distribution of Radiolabel in Male F344/N Rats 72 Hours after a Single Dermal Application of [^{14}C]-Trimethylolpropane Triacrylate[a]

	1.7 mg/kg[b]	15.2 mg/kg	130 mg/kg	151 mg/kg[c]
Absorbed Dose				
Urine	28.0 ± 1.2	12.1 ± 1.4	3.0 ± 0.4	5.8 ± 1.5
Cage wash	0.9 ± 0.4	0.9 ± 0.4	0.1 ± 0.0	0.4 ± 0.2
Feces	2.51 ± 0.5	1.2 ± 0.2	0.2 ± 0.1	0.4 ± 0.2
Exhaled CO_2	13.1 ± 1.4	4.9 ± 0.8	1.4 ± 0.2	3.4 ± 1.1
Dose site skin				
Acetone extractable	—[d]	8.0 ± 2.3	10.4 ± 5.1	8.5 ± 1.0
Nonextractable	8.5 ± 1.4	3.3 ± 0.6	3.2 ± 1.3	2.6 ± 0.6
Selected tissues	0.4 ± 0.1	1.0 ± 0.1	0.2 ± 0.0	0.7 ± 0.3
Residual carcass	1.7 ± 0.3	1.2 ± 0.2	0.3 ± 0.0	3.6 ± 2.3
Total Absorbed Dose	55.1 ± 3.0	32.7 ± 4.6	18.7 ± 6.7	25.4 ± 4.4
Total Unabsorbed Dose[e]	34.8 ± 4.8	57.0 ± 5.2	76.1 ± 6.3	65.3 ± 6.4
Total Dose Recovery	90.0 ± 2.9	89.7 ± 1.3	94.8 ± 2.3	90.7 ± 2.2

[a] Data are presented as percentage of dose (mean ± standard deviation) for five animals.
[b] n=4
[c] Animals were preexposed to a single dermal dose of 151 mg/kg unlabeled trimethylolpropane triacrylate 24 hours before administration of the radiolabeled dose.
[d] Skin samples not extracted
[e] Total radioactivity in appliance and skin wash

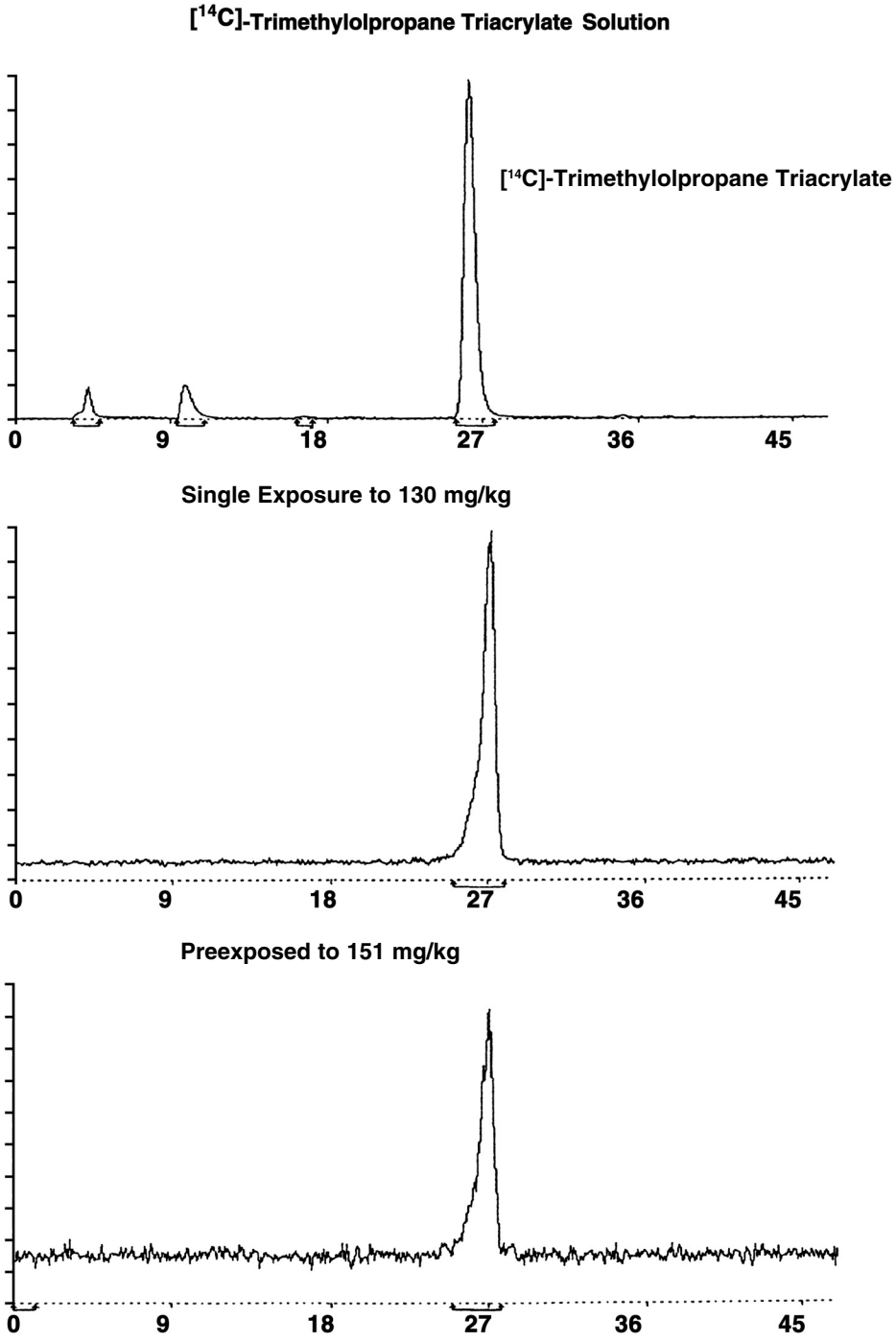

FIGURE L1
High-Performance Liquid Chromatographic Analysis of [^{14}C]-Trimethylolpropane Triacrylate and Acetone Extracts of Dose Site Skin 72 Hours after a Single Dermal Application of 130 or 151 mg/kg [^{14}C]-Trimethylolpropane Triacrylate to Male F344/N Rats
Animals dosed with 151 mg/kg were preexposed to a single dermal application of 151 mg/kg unlabeled trimethylolpropane triacrylate.

TABLE L2
Cumulative Excretion of Radiolabel by Male F344/N Rats after a Single Dermal Application of [^{14}C]-Trimethylolpropane Triacrylate[a]

Time (hours after dosing)	Urine	Feces	Exhaled CO_2	Cage Rinse
1.7 mg/kg[b]				
8	2.3 ± 0.8	0.0 ± 0.0	2.1 ± 0.4	—[c]
24	10.6 ± 0.9	0.9 ± 0.2	6.6 ± 0.8	—
48	21.1 ± 0.8	1.9 ± 0.4	10.6 ± 1.1	—
72	28.0 ± 1.2	2.5 ± 0.5	13.1 ± 1.4	0.9 ± 0.4
Total[d]		44.6 ± 2.7		
15.2 mg/kg				
8	0.5 ± 0.1	0.0 ± 0.0	0.4 ± 0.6	—
24	3.1 ± 0.3	0.3 ± 0.1	1.5 ± 0.2	—
48	7.2 ± 1.1	0.8 ± 0.1	3.1 ± 0.5	—
72	12.1 ± 1.4	1.2 ± 0.2	4.9 ± 0.8	0.9 ± 0.4
Total		19.2 ± 2.5		
130 mg/kg				
8	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	—
24	0.6 ± 0.2	0.0 ± 0.0	0.5 ± 0.0	—
48	1.7 ± 0.2	0.1 ± 0.0	0.9 ± 0.2	—
72	3.0 ± 0.4	0.2 ± 0.1	1.4 ± 0.2	0.1 ± 0.0
Total		4.7 ± 0.6		
151 mg/kg[e]				
8	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	—
24	1.0 ± 0.2	0.1 ± 0.0	0.5 ± 0.1	—
48	3.0 ± 0.8	0.2 ± 0.1	1.7 ± 0.4	—
72	5.8 ± 1.5	0.4 ± 0.2	3.5 ± 1.1	0.4 ± 0.2
Total		10.1 ± 2.8		

[a] Data are presented as cumulative percentage of dose (mean ± standard deviation) for five animals.
[b] n=4
[c] Samples not collected at this time point
[d] 72-Hour cumulative total of excreted radiolabel
[e] Animals were preexposed to a single dermal dose of 151 mg/kg unlabeled trimethylolpropane triacrylate 24 hours before administration of the radiolabeled dose.

TABLE L3
Tissue Distribution of Radiolabel in Male F344/N Rats 72 Hours after a Single Dermal Application of [^{14}C]-Trimethylolpropane Triacrylate[a]

Tissue	Trimethylolpropane Triacrylate Equivalents in Tissue (ng/g)	Tissue-to-Blood Ratio	Dose in Total Tissue[b] (%)
1.7 mg/kg[c]			
Adipose	11 ± 3	0.11 ± 0.05	0.04 ± 0.01
Bladder	89 ± 52	0.92 ± 0.64	0.00 ± 0.00
Blood	104 ± 29	—[d]	0.30 ± 0.09
Brain	7 ± 1	0.07 ± 0.02	0.00 ± 0.00
Heart	43 ± 21	0.40 ± 0.08	0.01 ± 0.01
Kidney	500 ± 86	4.98 ± 1.02	0.20 ± 0.04
Liver	66 ± 8	0.66 ± 0.14	0.12 ± 0.02
Lung	67 ± 31	0.63 ± 0.12	0.02 ± 0.01
Muscle	8 ± 1	0.08 ± 0.03	0.20 ± 0.03
Skin	22 ± 3	0.22 ± 0.03	0.20 ± 0.03
Spleen	69 ± 20	0.67 ± 0.13	0.01 ± 0.00
Testis	10 ± 2	0.11 ± 0.03	0.01 ± 0.00
15.2 mg/kg			
Adipose	75 ± 45	0.14 ± 0.10	0.03 ± 0.02
Bladder	406 ± 194	0.75 ± 0.31	0.00 ± 0.00
Blood	533 ± 6	—	0.18 ± 0.02
Brain	62 ± 47	0.11 ± 0.07	0.00 ± 0.00
Heart	255 ± 14	0.48 ± 0.03	0.01 ± 0.00
Kidney	5,910 ± 715	11.1 ± 0.9	0.28 ± 0.03
Liver	480 ± 47	0.90 ± 0.06	0.12 ± 0.01
Lung	321 ± 40	0.60 ± 0.03	0.01 ± 0.00
Muscle	53 ± 10	0.10 ± 0.02	0.17 ± 0.03
Skin	326 ± 40	0.61 ± 0.02	0.00 ± 0.00
Spleen	166 ± 8	0.31 ± 0.03	0.19 ± 0.01
Testis	114 ± 46	0.21 ± 0.08	0.01 ± 0.00
130 mg/kg			
Adipose	94 ± 35	0.08 ± 0.04	0.01 ± 0.00
Bladder	1,705 ± 810	1.49 ± 0.76	0.00 ± 0.00
Blood	1,163 ± 116	—	0.01 ± 0.01
Brain	78 ± 11	0.07 ± 0.01	0.00 ± 0.00
Heart	468 ± 46	0.40 ± 0.03	0.00 ± 0.00
Kidney	9,636 ± 2,334	8.34 ± 2.16	0.05 ± 0.01
Liver	1,201 ± 225	1.04 ± 0.24	0.03 ± 0.01
Lung	668 ± 104	0.58 ± 0.09	0.00 ± 0.00
Muscle	125 ± 37	0.11 ± 0.04	0.05 ± 0.01
Skin	324 ± 101	0.29 ± 0.12	0.04 ± 0.01
Spleen	698 ± 164	0.60 ± 0.12	0.00 ± 0.00
Testis	196 ± 127	0.18 ± 0.13	0.00 ± 0.00

TABLE L3
Tissue Distribution of Radiolabel in Male F344/N Rats 72 Hours after a Single Dermal Application of [^{14}C]-Trimethylolpropane Triacrylate

Tissue	Trimethylolpropane Triacrylate Equivalents in Tissue (ng/g)	Tissue-to-Blood Ratio	Dose in Total Tissue (%)
151 mg/kg[e]			
Adipose	863 ± 614	0.12 ± 0.05	0.04 ± 0.03
Bladder	6,441 ± 2,780	1.24 ± 1.11	0.00 ± 0.00
Blood	6,802 ± 2,776	—	0.09 ± 0.06
Brain	434 ± 154	0.07 ± 0.01	0.00 ± 0.00
Heart	2,655 ± 1,560	0.38 ± 0.13	0.01 ± 0.00
Kidney	20,098 ± 3,447	3.29 ± 1.08	0.09 ± 0.02
Liver	3,849 ± 1,384	0.59 ± 0.08	0.08 ± 0.03
Lung	3,807 ± 1,332	0.58 ± 0.06	0.02 ± 0.01
Muscle	500 ± 228	0.08 ± 0.04	0.16 ± 0.07
Skin	1,630 ± 1,282	0.23 ± 0.11	0.18 ± 0.14
Spleen	3,877 ± 1,477	0.58 ± 0.06	0.01 ± 0.00
Testis	674 ± 393	0.10 ± 0.03	0.00 ± 0.00

[a] Data are presented as mean ± standard deviation for five animals.
[b] Percent dose was calculated using the following values for the mass of total tissue, expressed as a percent of body weight: adipose, 7.0%; blood, 5.2%; muscle, 48%; and skin, 17%.
[c] n=4
[d] Unity
[e] Animals were preexposed to a single dermal dose of 151 mg/kg unlabeled trimethylolpropane triacrylate 24 hours before administration of the radiolabeled dose.

TABLE L4
Distribution of Radiolabel in Male B6C3F$_1$ Mice 72 Hours after a Single Dermal Application of 1.2 mg/kg [^{14}C]-Trimethylolpropane Triacrylate[a]

	Percentage of Dose
Absorbed Dose	
Urine	16.5 ± 2.8
Cage wash	2.0 ± 0.9
Feces	5.6 ± 4.2
Exhaled CO_2	18.2 ± 5.4
Dose site skin[b]	30.8 ± 4.9
Selected tissues	0.2 ± 0.1
Residual carcass	1.7 ± 0.2
Total Absorbed Dose	75.0 ± 2.4
Total Unabsorbed Dose[c]	20.9 ± 0.8
Total Dose Recovery	95.9 ± 1.8

[a] Data are presented as mean ± standard deviation for five animals.
[b] Skin samples were not extracted with acetone.
[c] Total radioactivity in appliance and skin wash

TABLE L5
Cumulative Excretion of Radiolabel by Male B6C3F$_1$ Mice after a Single Dermal Application of 1.2 mg/kg [^{14}C]-Trimethylolpropane Triacrylate[a]

Time (hours after dosing)	Urine	Feces	Exhaled CO_2	Cage Rinse
8	0.8 ± 1.6	—[b]	8.3 ± 2.3	—
24	10.0 ± 2.5	2.9 ± 2.6	13.7 ± 4.0	—
48	14.3 ± 2.9	4.3 ± 3.7	16.7 ± 4.9	—
72	16.5 ± 2.8	5.6 ± 4.2	18.2 ± 5.4	2.0 ± 0.9
Total[c]		42.4 ± 7.3		

[a] Data are presented as cumulative percentage of dose (mean ± standard deviation) for five animals.
[b] Samples not collected at this time point
[c] 72-Hour cumulative total of excreted radiolabel

TABLE L6
Tissue Distribution of Radiolabel in Male B6C3F$_1$ Mice 72 Hours after a Single Dermal Application of 1.2 mg/kg [^{14}C]-Trimethylolpropane Triacrylate[a]

Tissue	Trimethylolpropane Triacrylate Equivalents in Tissue (ng-Eq/g)	Tissue-to-Blood Ratio	Dose in Total Tissue (%)[b]
Adipose	3 ± 3	0.14 ± 0.12	0.02 ± 0.02
Bladder	35 ± 16	2.07 ± 1.55	0.00 ± 0.00
Blood	20 ± 6	—[c]	0.12 ± 0.02
Brain	2 ± 1	0.09 ± 0.04	0.00 ± 0.00
Heart	8 ± 1	0.44 ± 0.14	0.00 ± 0.00
Kidney	36 ± 3	1.93 ± 0.55	0.05 ± 0.01
Liver	20 ± 4	1.04 ± 0.27	0.07 ± 0.02
Lung	18 ± 11	0.88 ± 0.50	0.01 ± 0.00
Muscle	4 ± 1	0.20 ± 0.08	0.13 ± 0.04
Skin	106 ± 23	5.52 ± 1.65	1.20 ± 0.31
Spleen	11 ± 2	0.56 ± 0.09	0.00 ± 0.00
Testis	4 ± 1	0.22 ± 0.04	0.00 ± 0.00

[a] Data are presented as mean ± standard deviation for five animals.
[b] Percent dose was calculated using the following values for the mass of total tissue, expressed as a percent of body weight: adipose, 9.6%; blood, 7.6%; muscle, 45.2%; and skin, 14.4%.
[c] Unity

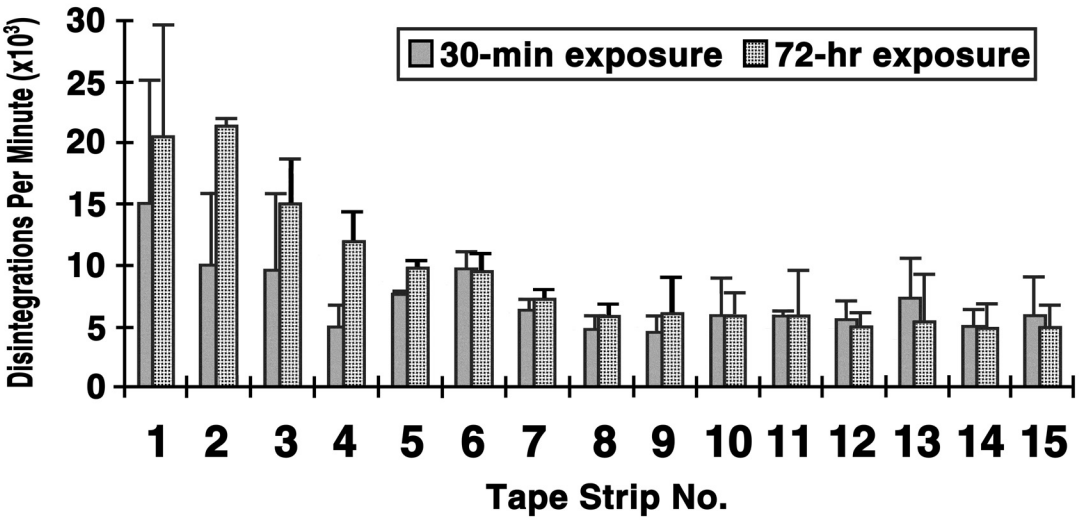

FIGURE L2
Radioactivity Present in Tape Strips of the Dermal Dose Sites 30 Minutes or 72 Hours after a Single Dermal Application of 124 mg/kg [^{14}C]-Trimethylolpropane Triacrylate to Male F344/N Rats
Values are mean ± standard deviation for four animals.

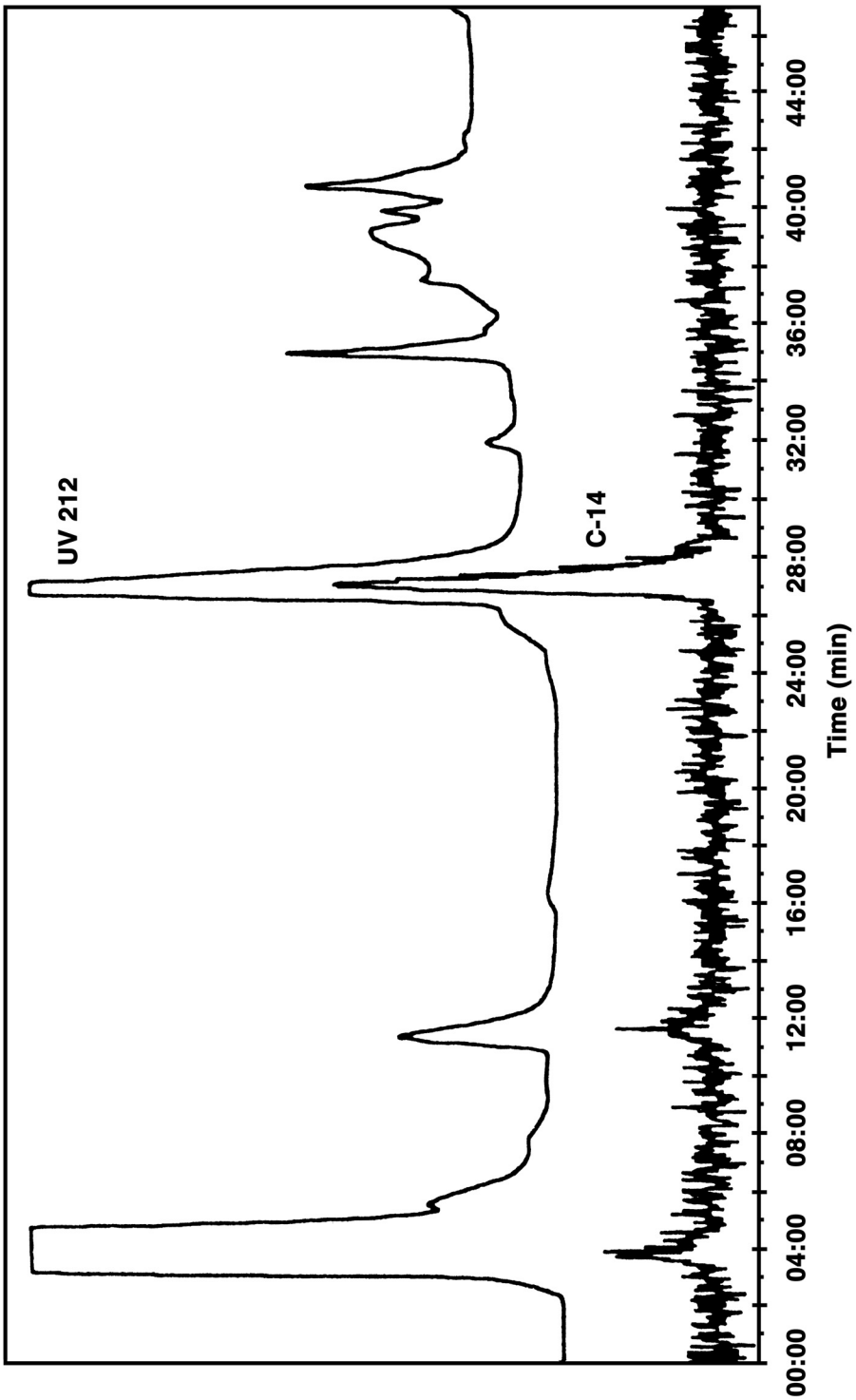

FIGURE L3
High-Performance Liquid Chromatographic Analysis of an Acetone Extract of the Stripped Dose Skin 72 Hours after a Single Dermal Application of 124 mg/kg [^{14}C]-Trimethylolpropane Triacrylate to Male F344/N Rats

TABLE L7
Cumulative Excretion of Radiolabel by Male F344/N Rats after a Single Intravenous Injection of 9.4 mg/kg [^{14}C]-Trimethylolpropane Triacrylate[a]

Time (hours after dosing)	Urine	Feces	Exhaled CO_2	Cage Rinse
3	—[b]	—	6.4 ± 0.8	—
6	24.1 ± 2.6	6.1 ± 5.5	12.9 ± 4.7	—
24	44.6 ± 4.9	6.1 ± 5.5	18.6 ± 5.9	—
48	47.3 ± 4.5	8.1 ± 7.0	19.8 ± 5.9	—
72	48.3 ± 4.4	8.7 ± 7.1	20.1 ± 6.0	0.3 ± 0.1
Total[c]		77.4 ± 3.5		

[a] Data are presented as cumulative percentage of the dose (mean ± standard deviation) for five animals.
[b] Samples not collected at this time point
[c] 72-Hour cumulative total of excreted radiolabel

TABLE L8
Concentration of Trimethylolpropane Triacrylate Equivalents in the Blood of Male F344/N Rats after a Single Intravenous Injection of 9.4 mg/kg [^{14}C]-Trimethylolpropane Triacrylate[a]

Time (hours after dosing)	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean ± Standard Deviation
0.08	15.2	24.3	39.7	27.0	33.5	27.9 ± 9.3
0.5	20.2	21.7	25.2	18.6	21.2	21.4 ± 2.4
1	12.9	22.0	16.9	10.8	19.7	16.5 ± 4.6
3	23.0	21.9	16.5	15.9	17.1	18.9 ± 3.3
6	22.1	20.7	20.0	14.9	16.6	18.9 ± 3.0
24	20.4	18.7	19.5	14.3	16.4	17.9 ± 2.5
48	17.6	17.1	17.0	12.6	15.4	15.9 ± 2.0
72	14.8	15.0	14.8	11.3	13.4	13.9 ± 1.6

[a] Data are presented as µg equivalents per gram of blood.

TABLE L9
Tissue Distribution of Radiolabel in Male F344/N Rats 72 Hours after a Single Intravenous Injection of 9.4 mg/kg [^{14}C]-Trimethylolpropane Triacrylate[a]

Tissue	Trimethylolpropane Triacrylate Equivalents in Tissue (ng-Eq/g)	Tissue-to-Blood Ratio	Dose in Total Tissue (%)[b]
Adipose	156 ± 47	0.01 ± 0.00	0.12 ± 0.04
Bladder	436 ± 80	0.03 ± 0.01	0.00 ± 0.00
Blood	13,800 ± 1,560	—[c]	5.01 ± 0.70
Brain	935 ± 370	0.07 ± 0.03	0.07 ± 0.03
Heart	3,880 ± 700	0.28 ± 0.05	0.12 ± 0.03
Kidney	2,530 ± 368	0.19 ± 0.04	0.20 ± 0.02
Liver	2,390 ± 189	0.17 ± 0.01	0.93 ± 0.17
Lung	9,570 ± 2,050	0.70 ± 0.17	0.50 ± 0.18
Muscle	173 ± 31	0.01 ± 0.00	0.81 ± 0.26
Skin	276 ± 31	0.02 ± 0.00	0.49 ± 0.06
Spleen	7,620 ± 1,150	0.55 ± 0.06	0.20 ± 0.04
Testis	142 ± 26	0.01 ± 0.00	0.02 ± 0.00

[a] Data are presented as mean ± standard deviation for five animals.
[b] Percent dose was calculated using the following values for the mass of total tissue, expressed as a percent of body weight: adipose, 7.0%; blood, 5.2%; muscle, 48%; and skin, 17%.
[c] Unity

TABLE L10
Distribution of Radiolabel in Male F344/N Rats 72 Hours after a Single Intravenous Injection of 9.4 mg/kg [^{14}C]-Trimethylolpropane Triacrylate[a]

Compartment	Cumulative % of Dose
Residual carcass	3.63 ± 0.2
Tail	1.07 ± 0.6
Selected tissues	2.04 ± 0.3
Urine	48.6 ± 4.5
Feces	8.7 ± 7.1
Exhaled CO_2	20.1 ± 6.0
72-hour blood	5.01 ± 0.7
Blood samples for assay	0.99 ± 0.2
Total	90.1 ± 2.6

[a] Data are presented as mean ± standard deviation for five animals.

Table L11
Binding of Radiolabel to Kidney Protein 72 Hours after a Single Dermal Application or Intravenous Injection of [^{14}C]-Trimethylolpropane Triacrylate in Male F344/N Rats

Route	Dose Level (mg/kg)	Kidney Radiolabel in Repetitive Extracts (%)								Total Radiolabel in Supernatants (%)	Total Radiolabel Remaining in Pellet (Bound) (%)	Pellet Suspension Protein Concentration (µg/mL)
		Trichloroacetic Acid[a]				Methanol:Water (80:20)						
		1	2	3	4	5	6	7	8			
Dermal	15.2	81.1	10.0	1.3	0.2	1.0	0.3	—[b]	—	93.9	6.1	65.3
Dermal	15.2	83.8	9.8	1.1	0.2	0.7	0.2	—	—	95.8	4.4	108
Intravenous	9.4	26.2	3.8	1.2	0.7	4.4	1.9	1.2	0.9	40.3	59.7	78.7

[a] 0.6 M Trichloroacetic acid final concentration
[b] Not extracted further