NTP REPORT

ON THE

TOXICOLOGY STUDIES OF

PENTAERYTHRITOL TRIACRYLATE

(Technical Grade)
(CAS NO. 3524-68-3)

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 4

NIH Publication No. 06-4451

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

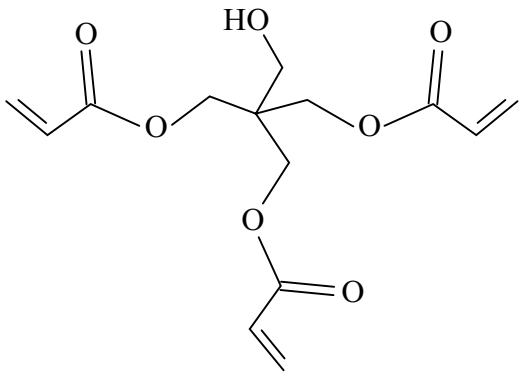
Background
Pentaerythritol triacrylate is used in a variety of photoreactive products including inks and coatings. People are exposed to pentaerythritol triacrylate mainly by skin contact. We used a genetically modified strain of mouse with sensitive skin to test if pentaerythritol triacrylate might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

Methods
We painted solutions of pentaerythritol 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 pentaerythritol triacrylate per kilogram body weight. Animals painted with acetone alone served as control groups. Tissues from 15 sites were examined for every animal.

Results
Almost all 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, squamous cell papilloma, and squamous cell carcinoma.

Conclusions
We conclude that pentaerythritol triacrylate caused skin papillomas in the genetically modified mouse model used in these studies.

ABSTRACT



PENTAERYTHRITOL TRIACRYLATE

CAS No. 3524-68-3

Chemical Formula: $C_{14}H_{18}O_7$ Molecular Weight: 298.3

Synonyms: Acrylic acid, pentaerythritol triester; pentaerythrityl triacrylate; PETA; 2-propenoic acid, 2-(hydroxymethyl)-2-(((1-oxo-2-propenyl)oxy)methyl)-1,3-propanediyl ester; tetramethylolmethane triacrylate
Trade names: Aronix M 305, NK Ester A-TMM3, Setalux UV 2242, SR 444, Viscoat 300

Pentaerythritol triacrylate is used in the production of ultraviolet-curable inks and coatings, electron beam irradiation-curable coatings, and radiation-cured and photocurable coatings of urethanes and epoxy resins; as a component of photopolymer and flexographic printing inks and plates and photoresists; as an ingredient of acrylic glues, adhesives, and anaerobic sealants; and as a modifier for polyester and fiberglass. It is also used in colloidal dispersions for industrial baked coatings, waterborne and solvent-based alkyds, vinyl/acrylic nonwoven binders, paper and wood impregnates, wire and cable extrusion, polymer-impregnated concrete, and polymer concrete structural composites. Pentaerythritol triacrylate was nominated by the National Cancer Institute for testing based on its high production volume and use, its potential for human exposure, and a lack of adequate testing of the chemical. Male and female F344/N rats and B6C3F$_1$ mice were administered technical grade pentaerythritol triacrylate (it is reactive and therefore not available as pure pentaerythritol triacrylate) in acetone dermally for 2 weeks or 3 months. Male and female Tg.AC hemizygous mice were administered technical grade pentaerythritol acrylate in acetone for 6 months. Genetic toxicology was evaluated in *Salmonella typhimurium* and 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 pentaerythritol triacrylate/kg body weight in acetone 5 days per week for 17 days. All rats survived to the end of the study; mean body weights of males administered 50 mg/kg or greater and 200 mg/kg females were significantly less than those of the vehicle controls. Irritation at the site of application occurred in all dosed groups except 12.5 mg/kg females. Epidermal hyperplasia, hyperkeratosis, sebaceous gland hyperplasia, ulcer, epidermal degeneration, parakeratosis, chronic active inflammation, and suppurative inflammation occurred at the site of application in most dosed groups of rats.

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 pentaerythritol triacrylate/kg body weight in acetone 5 days per week for 17 days. All mice survived to the end of the study. The final mean body weight and body weight gain of 25 mg/kg males were significantly greater than those of the vehicle controls, as was the mean body weight gain of 50 mg/kg males. All dosed groups had irritation at the site of application. Thymus weights of males administered 50 mg/kg or greater and 200 mg/kg females were significantly less than those of the vehicle controls. Most dosed groups of mice had epidermal hyperplasia, hyperkeratosis, sebaceous gland hyperplasia, ulcer, epidermal degeneration, parakeratosis, chronic active inflammation, and suppurative inflammation at the site of application.

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 pentaerythritol triacrylate/kg body weight in acetone 5 days per week for 14 weeks. All rats survived to the end of the study. Mean body weights of 12 mg/kg males were significantly less than those of the vehicle controls. Irritation at the site of application occurred in 12 mg/kg rats. Thymus weights of males administered 3 mg/kg or greater were significantly less than those of the vehicle controls. Hematology results indicated that pentaerythritol triacrylate induced a neutrophil count increase that would be consistent with an inflammatory response related to the dermatitis observed histopathologically. Epidermal hyperplasia, hyperkeratosis, epidermal degeneration and necrosis, chronic active inflammation, and sebaceous gland hyperplasia generally occurred at the application site in male and female groups administered 1.5 mg/kg or greater.

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 pentaerythritol triacrylate/kg body weight in acetone 5 days per week for 14 weeks. One female vehicle control mouse was sacrificed during the first week of the study due to ataxia and one 1.5 mg/kg female died during week 8. Mean body weights of dosed groups were similar to those of the vehicle control groups. Irritation at the site of application occurred in the 6 and 12 mg/kg male groups. Hematology results indicated an increased neutrophil count consistent with an inflammatory response related to the dermatitis observed histopathologically. There also was a minimal decrease in the erythron (hematocrit, hemoglobin concentration, and erythrocyte count) likely secondary to the inflammatory skin process. Males and females administered 1.5 mg/kg or greater generally had increased incidences of epidermal hyperplasia, degeneration, and necrosis; dermal chronic active inflammation, sebaceous gland hyperplasia, and hyperkeratosis at the site of application.

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 pentaerythritol triacrylate per kg body weight in acetone 5 days per week for 27 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. Survival of all dosed groups of mice was similar to that of the vehicle controls. With the exception of the 3 mg/kg group, body weights of male mice were less than those of the vehicle controls during the last 3 to 6 weeks of the study. Females administered 3 mg/kg had generally reduced body weights during the last month of the study. Treatment-related clinical findings included papillomas at the site of application in males and females receiving 3 mg/kg or more; papillomas were also observed in one 1.5 mg/kg male.

Heart and liver weights of 12 mg/kg males were significantly greater than those of the vehicle controls. Lung weights of 6 and 12 mg/kg males and females were significantly decreased, as were thymus weights of 6 and 12 mg/kg females.

Squamous cell neoplasms at the site of application were associated with dermal application of pentaerythritol triacrylate. At 6 months, all 3 and 6 mg/kg males had squamous cell papilloma at the site of application, and the incidences of this neoplasm were significantly increased in males and females receiving 3 mg/kg or more. Squamous cell carcinomas at the site of application occurred in two 3 mg/kg males, three 12 mg/kg males, and one 12 mg/kg female.

Nonneoplastic lesions noted at the site of application in dosed mice included hyperkeratosis, chronic active inflammation, and epidermal hyperplasia. Incidences of hematopoietic cell proliferation were increased in various organs, including the liver of 12 mg/kg females, the spleen of 6 and 12 mg/kg males and females, and the mandibular lymph node of 12 mg/kg females. A hematopoietic disorder (myelodysplasia) occurred in 12 mg/kg males.

GENETIC TOXICOLOGY

Pentaerythritol triacrylate was not mutagenic in several strains of *S. typhimurium*, with or without hamster or rat liver S9 activation enzymes. No increase in the frequency of micronucleated erythrocytes was observed in peripheral blood samples from $B6C3F_1$ mice treated with pentaerythritol triacrylate by skin painting for 3 months. In contrast, similar treatment of female Tg.AC hemizygous mice for 6 months induced a significant increase in micronucleated erythrocytes; the increase in micronuclei seen in male Tg.AC hemizygous mice was judged to be equivocal.

CONTACT HYPERSENSITIVITY STUDIES

Studies were conducted with female BALB/c mice to evaluate the potential for pentaerythritol triacrylate to induce contact hypersensitization. In an irritancy study in which formulations of pentaerythritol triacrylate (approximately 10% or 45% pure) in acetone were applied to the ear, the maximal nonirritating concentration was 0.1% and the minimal irritating concentration was 0.25% for both mixtures. A mouse ear swelling test yielded negative results for pentaerythritol triacrylate as a potential contact sensitizer using the 10% mixture and positive results with the 45% mixture. Positive responses were seen in local lymph node assays at concentrations of 0.05%, 0.1%, and 0.25% pentaerythritol triacrylate when the approximately 10% pentaerythritol triacrylate mixture was used and at a concentration of 0.25% pentaerythritol triacrylate when the approximately 45% pentaerythritol triacrylate mixture was used.

CONCLUSIONS

Male and female Tg.AC hemizygous mice dosed with pentaerythritol triacrylate for 6 months had significantly increased incidences of squamous cell papillomas of the skin at the site of dermal application. Treatment-related squamous cell carcinomas occurred at the site of application in male mice.

Nonneoplastic lesions noted at the site of application included hyperkeratosis, chronic active inflammation, and epidermal hyperplasia. A hematopoietic disorder (myelodysplasia) occurred in dosed male mice.

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

	Male Tg.AC Mice	Female Tg.AC 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	All dosed groups except 3 mg/kg less than the vehicle controls during the last 3 to 6 weeks of the study	3 mg/kg groups generally less than vehicle controls during last month of study
Survival rates	12/15, 14/15, 15/15, 15/15, 12/15, 10/15	12/15, 14/15, 12/15, 12/15, 13/15, 9/15
Nonneoplastic effects	Skin (site of application): hyperkeratosis (1/15, 0/15, 2/15, 6/15, 10/15, 13/15); inflammation, chronic active (0/15, 0/15, 0/15, 2/15, 5/15, 12/15); epidermis, hyperplasia (0/15, 0/15, 3/15, 8/15, 10/15, 14/15) All organs: myelodysplasia (0/15, 0/15, 0/15, 0/15, 0/15, 5/15)	Skin (site of application): hyperkeratosis (1/15, 0/15, 4/15, 14/15, 11/15, 13/15); inflammation, chronic active (0/15, 0/15, 1/15, 9/15, 10/15, 15/15); epidermis, hyperplasia (0/15, 0/15, 5/15, 14/15, 14/15, 14/15)
Neoplastic effects	Skin (site of application): squamous cell papilloma (1/15, 0/15, 4/15, 15/15, 15/15, 13/15); squamous cell carcinoma (0/15, 0/15, 0/15, 2/15, 0/15, 3/15)	Skin (site of application): squamous cell papilloma (0/15, 0/15, 1/15, 10/15, 12/15, 13/15)
Genetic toxicology		
Salmonella typhimurium gene mutations:	Negative in strains TA100, TA1535, TA1537, and TA98, with and without S9	
Micronucleated erythrocytes		
Mouse peripheral blood *in vivo:*		
$B6C3F_1$	Negative in males and females	
Tg.AC hemizygous	Equivocal in males and positive in 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 pentaerythritol 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 Technical 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 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 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 papilloma of the skin at the site of chemical application in males and females, plus skin carcinoma 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 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 principal reviewer, felt that 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 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 than *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 (SAM), the use of monomeric forms of SAM in producing cross-linked polymers, and a series of industry studies on two representative chemicals from this group (triethylene glycol diacrylate and the corresponding methacrylate). He noted that skin irritation is characteristic of SAM, 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 that those measures were just of the monomer, whereas the technical grade material consisted of a mixture including oligomers and other monofunctional acrylates.

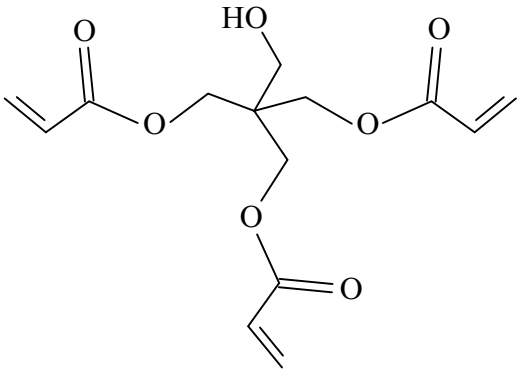
Dr. Drinkwater then turned the discussion to the general question of the use of transgenic models by the NTP, noting that suggestions have ranged from use as a preliminary screen to complete replacement of the conventional bioassay. Dr. Ho expressed optimism that the shorter time involved in the transgenic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



PENTAERYTHRITOL TRIACRYLATE

CAS No. 3524-68-3

Chemical Formula: $C_{14}H_{18}O_7$ Molecular Weight: 298.3

Synonyms: Acrylic acid, pentaerythritol triester; pentaerythrityl triacrylate; PETA; 2-propenoic acid, 2-(hydroxymethyl)-2-(((1-oxo-2-propenyl)oxy)methyl)-1,3-propanediyl ester; tetramethylolmethane triacrylate
Trade names: Aronix M 305, NK Ester A-TMM3, Setalux UV 2242; SR 444, Viscoat 300

CHEMICAL AND PHYSICAL PROPERTIES

Pentaerythritol triacrylate is a colorless or light amber nonvolatile liquid; it also occurs as a white semisolid or crystalline solid at temperatures up to 40° C (Celanese, 1979; AIHA, 1981; Lenga, 1988). It has a characteristic acrylate odor (Radcure, 1990a). Pentaerythritol triacrylate has a melting range from 25° to 40° C and a boiling point of greater than 315° C at 760 mm Hg (AIHA, 1981; Radcure, 1990b). At 100° C, it has a vapor pressure of less than 0.01 mm Hg (Radcure, 1990b); its refractive index at 20° C is 1.4864 (Lenga, 1988). Pentaerythritol triacrylate is practically insoluble in water; hygroscopic; and incompatible with strong oxidizing agents, strong acids, and strong bases (Lenga, 1988; Radcure, 1990a). Pentaerythritol triacrylate may polymerize when exposed to sources of free radicals (Radcure, 1990b), direct light, and heat; it is stabilized with 300 to 400 ppm hydroquinone monomethyl ether (*Aldrich*, 2000). Decomposition products include toxic fumes of carbon monoxide and carbon dioxide (Lenga, 1988; Radcure, 1990a). The chemical is reactive and is therefore not available as pure pentaerythritol triacrylate.

PRODUCTION, USE, AND HUMAN EXPOSURE

No information on the specific manufacturing process of pentaerythritol triacrylate was found in the literature. However, polyfunctional acrylate monomers can be produced by direct or *trans*-esterification methods (*Kirk-Othmer*, 1978).

Pentaerythritol is manufactured by the reaction of acetaldehyde with formaldehyde in alkaline medium such as sodium or calcium hydroxide (*Kirk-Othmer*, 1978). Initially, the alpha-hydrogen atoms of acetaldehyde condense with formaldehyde in three sequential aldol reactions to form pentaerythrose. Pentaerythrose is then reduced to pentaerythritol in a crossed Cannizzaro reaction with formaldehyde. Pentaerythritol esters have been synthesized by the usual methods of esterification using organic acids, acid anhydrides, or acid chlorides.

The U.S. Environmental Protection Agency's Toxic Substances Control Act Inventory for 1983 indicated that approximately 100,000 to 1 million pounds of

pentaerythritol triacrylate were produced by three U.S. companies providing annual production volumes (USEPA, 1991). The American Industrial Hygiene Association (1981) estimated the production of multifunctional acrylates including pentaerythritol triacrylate may be several million pounds per year. More recent production data were not available.

Pentaerythritol triacrylate is used in the production of ultraviolet-curable inks and coatings, electron beam irradiation-curable coatings, and radiation-cured and photocurable coatings of urethanes and epoxy resins; as a component of photopolymer and flexographic printing inks and plates and photoresists; as an ingredient of acrylic glues, adhesives, and anaerobic sealants; and as a modifier for polyester and fiberglass (Nethercott, 1978; Björkner, 1984; ACS, 1990; Newmark and Palazzotto, 1990). It is also used in colloidal dispersions for industrial baked coatings, waterborne and solvent-based alkyds, vinyl/acrylic nonwoven binders, paper and wood impregnates, wire and cable extrusion, polymer-impregnated concrete, and polymer concrete structural composites (Celanese,1982a; NCI, 1987).

Workers involved in the manufacturing, processing, product handling, and application of pentaerythritol triacrylate are at risk of exposure (Parker and Turk, 1983). Surveys by the National Institute for Occupational Safety and Health (1990) found that 62 workers were potentially exposed to pentaerythritol 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 pentaerythritol triacrylate/m^3. Consumers may potentially be exposed through the use of products such as latex paint and floor polishes that contain pentaerythritol triacrylate (Dearfield *et al.*, 1989). In addition, products that are made from high-impact acrylic molding powders containing pentaerythritol triacrylate include outboard motor shrouds, housings and containers, nameplates, toys, business machine components, and blow-molded bottles (NCI, 1987).

Absorption, Distribution, Metabolism, and Excretion

No studies on the absorption, distribution, metabolism, or excretion of pentaerythritol triacrylate in experimental animals or in humans were found in a review of the literature.

Toxicity

Experimental Animals

Acute oral LD_{50} values reported for rats were 2.46 mL pentaerythritol triacrylate/kg body weight (Carpenter *et al.*, 1974), 1,350 mg/kg (AIHA, 1981), or greater than 500 to 5,000 mg/kg (Andrews and Clary, 1986). Intraperitoneal injection LD_{50} values reported for rats ranged from 18.5 to 27 mg/kg (Celanese, Inc., 1982b). Dermal LD_{50} values for rabbits were 4 mL/kg (Carpenter *et al.*, 1974), greater than 2,000 mg/kg (AIHA, 1981), and greater than 200 to 2,000 mg/kg (Andrews and Clary, 1986).

The maximum time rats could be exposed to concentrated vapors of pentaerythritol triacrylate with no deaths was 8 hours (Carpenter *et al.*, 1974). Signs of neurological toxicity, including ataxia, flaccid limb and body tone, and abnormal righting and visual placing reflexes were seen in male and female albino Sprague-Dawley rats administered intraperitoneal injections of 10 to 100 mg/kg (Celanese, 1982c; NCI, 1987). Applications of this chemical to the eye of rabbits caused severe and corrosive irritation as well as corneal opacity (Carpenter *et al.*, 1974; Andrews and Clary, 1986), and 1 mg of pentaerythritol triacrylate applied to the eye of rabbits resulted in severe irritation (Lenga, 1988; Sax and Lewis, 1989).

A significant increase in lymph node weight was noted in outbred male and female Hartley guinea pigs 4 to 6 days after epicutaneous immunization with 50 µmol of pentaerythritol triacrylate (Bull *et al.*, 1985). Evidence of increased T-lymphocyte proliferation in the lymph node, as measured by an increase in the number of large pyroninophilic cells, was also observed. The authors concluded that a positive correlation exists among skin sensitization reactions, increased lymph node weights, and T-lymphocyte proliferation.

In a sensitization study by Nethercott (1978), 15 albino Hartley/Dalkin guinea pigs (sex not specified) were induced and then challenged with pentaerythritol triacrylate. Three intradermal injections were administered to each shoulder: 0.1 mL of 0.05% pentaerythritol triacrylate in propylene glycol, 0.05 mL of 0.1% pentaerythritol triacrylate in propylene glycol with 0.05 mL of Freund's complete adjuvant (FCA), and 0.1 mL FCA. After 1 week, 25% pentaerythritol 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 10% pentaerythritol triacrylate in petrolatum for 24 hours. Thirteen of the animals reacted positively to pentaerythritol triacrylate.

In another study, female albino Hartley/Dalkin guinea pigs received three pairs of intradermal injections of 0.001% to 0.5% pentaerythritol triacrylate in propylene glycol, 0.05 mL of FCA mixed with 0.05 mL of the appropriate concentration of pentaerythritol triacrylate, and 0.1 mL of FCA in the shoulder (Nethercott *et al.*, 1983). One week later, pentaerythritol triacrylate at the same concentrations was applied to the shaved shoulder via a filter paper patch; the patch was kept in place for 48 hours. The animals were challenged 2 weeks after topical exposure with 24-hour shaved-flank skin patches of nonirritant concentrations of pentaerythritol triacrylate. The percentage of animals sensitized at each concentration increased with exposure concentration, ranging from 15% of those receiving 0.001% pentaerythritol triacrylate to 100% of those receiving 0.5%.

The contact sensitization potential of pentaerythritol triacrylate was studied in male and female outbred Hartley guinea pigs using five different immunization protocols (Parker and Turk, 1983). On the first day of the Polak immunization method, animals were administered four footpad injections and one injection in the nape of the neck of a 0.1 mL emulsion containing 2 mg/mL pentaerythritol triacrylate in ethanol:saline (1:4) in FCA. On day 7, 0.02 mL of 0.1% or 0.25% pentaerythritol triacrylate in acetone:olive oil (4:1) was dropped onto the shaved flank of the animals. The skin tests were repeated weekly at different sites on the flank for up to 12 weeks. In the split adjuvant immunization method, 0.05 mL of FCA was injected into five sites on the dorsal shaved flank. One day later 0.1 mL pentaerythritol triacrylate in ethanol:saline (1:100) was injected intradermally into the same five sites. Skin tests as described for the Polak method were begun on day 14. In the maximization immunization method, guinea pigs received two intradermal injections of 0.1 mL FCA, 0.1 mL of 1% pentaerythritol triacrylate in saline, and 0.1 mL of 1% pentaerythritol triacrylate in FCA. Skin tests as described above were begun 14 days later. In the first epicutaneous method, 0.1 mL of a 0.3 M solution of pentaerythritol triacrylate in 95% ethanol:2-methoxyethanol:Tween 80 (9:9:2) was dropped once daily onto the animals' shaved flank on days 1, 3, 5, 8, 10, and 12. On day 29, weekly skin testing as described above was begun. In the other epicutaneous method, 0.1 mL of 0.25% pentaerythritol triacrylate in acetone:olive oil (1:1) was applied once daily to a shaved area on the back of the neck for 5 days and again on days 8 through 12. The skin test procedure as described above began on day 22. In all methods, skin test sites were observed 24, 48, and 72 hours after application. Six guinea pigs exhibited positive skin reactions on the 14th day with the Polak method; reactions were graded on a scale of 0 (no reaction) to 3 (red and elevated reaction). Scores of 1.1 using 0.1% pentaerythritol triacrylate and 1.4 using 0.25% pentaerythritol triacrylate were reported. With the second epicutaneous method, five of six animals were sensitized; the severity of these reactions was not reported.

In a study to determine whether animals sensitized with trimethylolpropane triacrylate would exhibit cross-sensitivity when challenged with other multifunctional acrylates, albino female Dunkin Hartley guinea pigs were given three pairs of intradermal injections in the shoulder: 0.1 mL of FCA mixed 1:1 with water, 0.1 mL of 1% trimethylolpropane triacrylate in olive oil, and 0.1 mL of 1% trimethylolpropane triacrylate in olive oil with an equal amount of FCA (Björkner *et al.*, 1980). After 1 week, a shoulder patch of 25% trimethylolpropane triacrylate in petrolatum was applied for 48 hours. Two weeks after this induction procedure, groups of 24 animals were challenged with occlusive patch tests of 0.5% or 0.1% pentaerythritol triacrylate in petrolatum applied to a shaved area of the flank for 24 hours. Twelve animals (50%) sensitized to trimethylolpropane triacrylate had positive reactions when challenged with 0.1% pentaerythritol triacrylate; 18 animals (75%) reacted positively to the 0.5% concentration, suggesting cross-sensitivity between the two compounds.

In a maximization test to determine skin sensitivity and cross-sensitivity reactions, groups of 15 female albino Dunkin Hartley guinea pigs were sensitized with intradermal injections of 1% commercial-grade pentaerythritol tri- or tetraacrylate in olive oil:acetone (9:1) followed by topical patches of 25% solutions of these chemicals in petrolatum and then challenged with two applications on the flank, 1 week apart, of 0.015 g of the test acrylate or trimethylolpropane triacrylate in petrolatum (Björkner, 1984). A 1% 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. It was concluded from these results that pentaerythritol triacrylate was the more potent sensitizer and that guinea pigs sensitized to pentaerythritol triacrylate may cross-react to trimethylolpropane triacrylate.

Bull *et al.* (1985) immunized outbred male and female Hartley guinea pigs (number not specified) with subcutaneous injection in the footpads and in the neck with 0.1 mL of an emulsion of pentaerythritol triacrylate in ethanol:saline (4:1). The total pentaerythritol triacrylate dose was approximately 11.5 µmol. In addition, five guinea pigs were sensitized by applying 50 µL of a 1M solution of pentaerythritol triacrylate in acetone:olive oil (4:1) to the dorsum of each animal's right ear. Skin tests of 0.02 mL of 0.1% or 0.25% pentaerythritol triacrylate in acetone:olive oil (4:1) were applied to the shaved flank of the guinea pig one and two weeks after induction, and reactions were recorded at 24, 48, 72, and 96 hours. Immunization with pentaerythritol triacrylate by the Polak method resulted in positive skin reactions; sensitization was not induced by epicutaneous application.

Parker *et al.* (1985) extended the Bull *et al.* (1985) study to examine the ability of pentaerythritol triacrylate to induce a tolerance to the contact reactions seen in guinea pigs. Groups of five male and female outbred Hartley guinea pigs were immunized with pentaerythritol triacrylate and skin tested with pentaerythritol triacrylate as well as with additional acrylates of similar structure, as described by Bull *et al.* (1985). To induce tolerance epicutaneously, 0.1 mL of 10% pentaerythritol triacrylate in acetone was applied to the dorsum of each animal's ear 14 and 7 days before immunization with methyl acrylate or trimethylolpropane triacrylate. Skin tests (methyl acrylate at 1% or 5%; trimethylolpropane triacrylate at 0.25% or 0.5%) were conducted with the immunizing compound 7 days after induction, and reactions were scored 24 and 48 hours after the patches were removed. Animals sensitized to pentaerythritol triacrylate also reacted to trimethylolpropane triacrylate, 4-vinyl pyridine, methyl acrylate, and methyl vinyl ketone, indicating that pentaerythritol triacrylate has the potential to cross-react with several other chemicals.

In another study to examine cross-reaction patterns of pentaerythritol triacrylate and other selected acrylates, female outbred SSc:AL guinea pigs were induced with three pairs of intradermal injections in the shoulder: 2 × 50 µL of FCA in sterile water (1:1); 2 × 50 µL of a 5% solution of the test chemical in soybean oil, and 2 × 50 µL of a 5% solution of the test chemical in FCA and water (1:1) (Clemmensen, 1984). On day 8, 250 mg of 10% sodium dodecyl sulfate in petrolatum was applied to the same site and left uncovered for 24 hours. On day 9, 400 µL of the test compound (100%) was applied to the test area on a patch held in place for 48 hours. Challenge patch tests were conducted on day 22 with up to six patches containing 25 µL 2% pentaerythritol triacrylate that were held on a shaved area of the animal's flank for 24 hours. Reactions were graded 48 and 72 hours after the application of the patches. Seven of 20 animals that had been induced with triethyleneglycol dimethacrylate and nine of 19 animals induced with trimethylolpropane trimethacrylate reacted to pentaerythritol triacrylate. No cross-reaction was seen in animals induced with methylmethacrylate or ethyleneglycol dimethacrylate. The author concluded that pentaerythritol triacrylate is a potent sensitizer that can cross-react with other acrylates.

Groups of five male and five female New Zealand White rabbits received unoccluded dermal applications of 200 mg pentaerythritol triacrylate/kg body weight at a site on the back, 5 days a week for 2 weeks (Celanese, 1979; NCI, 1987). For five animals, the exposure sites were further treated by abrading the area with an inverted clipper before treatment and twice weekly thereafter. Three abraded and three nonabraded animals of each sex per group were sacrificed following the last day of treatment; the remaining animals were sacrificed after day 30. All animals treated with pentaerythritol triacrylate exhibited severe erythema with necrosis and eschar formation, fissuring, desquamation, and slight to moderate edema and atonia at 2 weeks; erythema and desquamation persisted through week 4. Treated animals also had discoloration of the lung and kidney at 2 weeks; these abnormalities were not present at week 4. Microscopically, dosed animals had severe necrosis of the epithelium and subepithelium and congestion of the dermis at 2 weeks. The epithelium was intact at 4 weeks, but there was residual evidence of earlier irritation. No evidence of systemic toxicity resulting from administration of pentaerythritol triacrylate was observed. In a similar study, with no recovery period, in which the animals were dermally administered 500 mg/kg per day for 2 weeks, six animals died during the treatment period, and all dosed animals lost weight during the study (Celanese, 1981;

NCI, 1987). At day 7, all dosed animals exhibited moderate to severe erythema and edema. At the time of death (spontaneous or sacrifice), most animals exhibited necrosis, eschar formation, and atonia; a few also showed slight desquamation and/or fissuring. Histologic examination of dosed rabbits revealed extensive degeneration of the subcutis and severe epidermal necrosis and ulceration in dosed animals sacrificed at 2 weeks. All six animals that died during the study had degeneration and edema of the dermis and degeneration and inflammation of the subcutis; epidermal necrosis was seen in five of these animals.

Humans

Pentaerythritol triacrylate has been shown to be a cutaneous sensitizer in humans. For induction, patches containing a 10% solution of pentaerythritol triacrylate were applied on the extensor surface of the arm of eight human volunteers ages 18 to 25 (Nethercott, 1978). The patch was removed each day and reapplied if there was no evidence of inflammation. For the challenge test 4 weeks later, patches containing 0.1% pentaerythritol triacrylate in petrolatum were applied to the subjects' upper back for 48 hours. One subject had macular erythema, four exhibited a vesicular or oedematous reaction, and one subject had a severe spreading, ulcerative reaction.

A 61-year-old male painter developed eczematous dermatitis of the hands, arms, trunk, and legs within weeks after his company's switch from oil-based paint to an acrylic-based system (Cofield *et al.*, 1985). The new system contained 1% to 3% of a polyfunctional aziridine cross-linking hardener composed of a multifunctional acrylic monomer, trimethylolpropane triacrylate, at residual concentrations ranging from 0.03% to 0.15%. Skin patch tests using 0.2% pentaerythritol triacrylate, full strength paint primer (without cross-linker), and the full strength aziridine cross-linker, all in petrolatum, were performed. The patches were kept in place for 48 hours and the results were read 15 minutes after the patches were removed and again at one week. Positive reactions were seen to the pentaerythritol triacrylate and the full strength cross-linker at 48 hours and 1 week. Because the patient had been exposed only to a cross-linker composed of trimethylolpropane triacrylate, the reaction to pentaerythritol triacrylate could be attributed to cross-sensitization.

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). Although the hardener in this case was composed of trimethylolpropane triacrylate, pentaerythritol triacrylate can also be substituted as the hardener in the varnish. All four patients tested positive to skin patch tests with 0.1% hardener in acetone, and to a 0.1% solution of trimethylolpropane triacrylate in acetone. Two patients also reacted to 0.1% pentaerythritol triacrylate in acetone.

The addition of two new multifunctional acrylates, trimethylolpropane triacrylate and pentaerythritol triacrylate, as components of a radiation drying ink at an ink formulation facility, was associated with eczematous dermatitis in five of 26 employees (Emmett, 1977). Patch testing was done individually with each component of the ultraviolet-cured inks used in the plant, as wells as solutions of 0.2% pentaerythritol triacrylate in petrolatum and three ink varnish formulations containing 0.2% pentaerythritol triacrylate in petrolatum. Four of the five employees reacted positively to pentaerythritol triacrylate and the three varnish formulations containing pentaerythritol triacrylate. Although the fifth employee did not have significant reactions at 48 or 72 hours, he did exhibit an irritant dermatitis following patch testing with pentaerythritol triacrylate. All subjects sensitized to pentaerythritol triacrylate also reacted to trimethylolpropane triacrylate, a similar polyfunctional acrylate monomer. However, because all subjects had potential exposure to both compounds, it was unclear whether the multiple reactivity was a result of cross-sensitization or concomitant sensitization.

Of approximately 58 employees in one plant who were potentially exposed to an ultraviolet-cured ink manufacturing process, eight men ages 24 to 62 developed symptoms consistent with allergic contact dermatitis (Emmett and Kominsky, 1975, 1977). In six cases, the symptoms were seen 1 or 2 days after working with ultraviolet inks. Occlusive patch tests of 0.2% pentaerythritol triacrylate or trimethylolpropane triacrylate in petrolatum were conducted for 48 hours and the sites were examined 1 or 24 hours later. Four of the tested subjects had positive reactions to pentaerythritol triacrylate and, in three cases, the results were categorized as strong edematous or vesicular reactions. The authors concluded that pentaerythritol triacrylate is a strong sensitizing agent but cross-sensitization could not be evaluated in these subjects because the workers were potentially exposed to multiple agents.

In an ink manufacturing plant, 19 workers exposed to ultraviolet-cured inks developed skin and conjunctival reactions (Nethercott, 1978). Subjects were patch tested with several acrylate compounds, including a solution of 0.1% pentaerythritol triacrylate in petrolatum. Seven workers reacted positively to pentaerythritol triacrylate and were considered sensitized to the compound, which each had handled as a raw material.

Seven of 10 employees of a plastic food container manufacturing plant developed various cutaneous conditions, including eczematous dermatitis to erythematous scaling (Nethercott *et al.*, 1983). All seven were skin patch tested with several potential allergens, including urethane acrylate and pentaerythritol triacrylate (0.1% in petrolatum). Five of the seven employees tested positive to urethane acrylate and one showed a positive reaction to pentaerythritol triacrylate at both 48 and 96 hours. Because the chemical structures of these two compounds differ, the pentaerythritol triacrylate-responsive individual had been exposed to both substances, and chemical analysis showed no contamination of urethane acrylate with pentaerythritol triacrylate, the authors concluded these reactions represented concurrent sensitization rather than cross-sensitization.

A worker developed an erythematous pruritic rash on his hands and face months after being exposed to a new printing system that used ultraviolet-cured inks; the rash would disappear when he was not exposed to the inks (Smith, 1977). Another man reported similar skin eruptions. The men were skin patch tested with three colored inks (red, black, and gold) diluted to 10% in methyl ethyl ketone and with the reducer, pentaerythritol triacrylate. Positive reactions were seen with pentaerythritol triacrylate and the black and gold inks that contained pentaerythritol triacrylate. No reaction was seen with red ink, which did not contain pentaerythritol triacrylate. A second series of patch tests using pentaerythritol triacrylate at 0.1% and 1% in methyl ethyl ketone, the other components of the inks, and two alternative acrylates was performed. Both men had positive patch tests with pentaerythritol triacrylate and the two alternative acrylates, trimethylolpropane triacrylate and tripropylene glycol triacrylate, but not with any other ink component. The author concluded that pentaerythritol triacrylate is a strong allergen capable of producing sensitization; positive reactions observed with the other two acrylates were thought to be due to cross-sensitization.

Fifty-nine cases of skin disorders of the hands were reported after the use of protective gloves made from modified polyvinyl chloride after the addition of 2.2% pentaerythritol triacrylate to the gloves' formulation (Kalensky, 1987). Skin patch tests with 0.2% pentaerythritol triacrylate in alcohol produced intense allergic reactions and the concentration was reduced to 0.1% in Vaseline®. An annular reaction was seen in five subjects following patch testing with the 0.1% formulation. The author concluded that pentaerythritol triacrylate, a strong sensitizing agent, was unsuitable for use in protective gloves.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Uncommon malformations were seen in a small number of fetuses born to 20 female rats (strain unspecified) administered a single dermal dose of 100 mg pentaerythritol triacrylate/kg body weight during days 6 through 15 of gestation (Andrews and Clary, 1986). This dose had been determined to be the maximum at which slight maternal toxicity was observed. However, teratogenic effects were not noted in a second study in which pentaerythritol triacrylate was administered at an unspecified dose that caused minimal maternal toxicity. From these results, the authors concluded that pentaerythritol triacrylate was not a teratogen.

Humans

No information on the reproductive or developmental toxicity of pentaerythritol triacrylate in humans was found in the literature.

CARCINOGENICITY

Experimental Animals

Male C3H/HeJ mice treated with approximately 3 mg of a mixture of 25% pentaerythritol triacrylate, 65% tetraacrylate, and 10% diacrylate applied as a 15% solution in acetone three times per week to the back for life showed no incidence of skin tumors (DePass, 1982; DePass *et al.*, 1985).

No skin tumors were found in male C3H/HeJ mice treated with a 15% solution of pentaerythritol acrylate-HF (approximately 3 mg/mouse per application) on the back three times per week for the life of the animals (Union Carbide, 1979, 1988; NCI, 1987). Thirteen of 39 mice in the dosed group had gross hepatic tumors, but the increased incidence of these lesions was considered

to be equivocal when compared to historical control data.

A group of 50 male C3H/HeJ mice was treated twice weekly with 50 mg of 5% pentaerythritol triacrylate in white mineral oil (2.5 mg/mouse) painted onto the shaved interscapular region for up to 80 weeks (Celanese, 1986). One treated mouse developed a squamous cell carcinoma of the skin, and six mice had lymphomas with spleen or lymph node involvement. To determine the toxicologic significance of these findings, the U.S. Environmental Protection Agency reviewed these results and concluded that these lesions provided limited positive evidence of carcinogenicity of pentaerythritol triacrylate in C3H/HeJ mice (NCI, 1987).

Humans

No epidemiology studies or case reports associating pentaerythritol triacrylate exposure with cancer risk were found in a review of the literature.

Related Compounds

Pentaerythritol triacrylate is a member of the multifunctional alkyl acrylates class. 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 life of the animals (DePass *et al.*, 1985). Among mice administered neopentylglycol diacrylate, five had skin papilloma and three others had skin carcinoma. No skin neoplasms were observed in pentaerythritol triacrylate-treated mice.

Genetic Toxicity

Pentaerythritol triacrylate, in concentrations up to 10,000 µg/plate, was not mutagenic in any of several strains of *Salmonella typhimurium*, with or without metabolic activation (Zeiger *et al.*, 1987). It was tested in L5178Y mouse lymphoma $TK^{+/-}$ cells, in the absence of exogenous metabolic activation, for induction of forward mutations at the TK locus, chromosomal aberrations, and micronuclei (Dearfield *et al.*, 1989). Pentaerythritol triacrylate induced significant dose-related increases in all three endpoints measured in the latter study. The highest dose tested was 0.350 µg/mL, and at this dose, cell survival was reduced to 15%. No additional publications of mutagenicity test data for pentaerythritol triacrylate were found in a review of the literature.

Study Rationale

Pentaerythritol triacrylate was nominated for dermal carcinogenicity studies by the National Cancer Institute based on its high and increasing production volume, its widespread use and potential for human exposure, the lack of adequate chronic toxicity and carcinogenicity data, and the lack of adequate structure activity and genotoxicity data on the acrylate class of chemicals. As a member of the class of multifunctional acrylates, pentaerythritol triacrylate is a suspected carcinogen; some members of this class have been shown to be carcinogenic to mice in dermal studies.

The major route of human exposure to pentaerythritol 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

Pentaerythritol Triacrylate

Pentaerythritol triacrylate was obtained in two lots. Lot 05318JW, obtained from Aldrich Chemical Company (Milwaukee, WI), was used during the 2-week studies, and lot HCC0340, obtained from Sartomer Company (Exton, PA), was used during the 3- and 6-month 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 pentaerythritol triacrylate studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a clear, viscous liquid, was identified as pentaerythritol triacrylate using infrared spectroscopy and proton and carbon-13 nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure of pentaerythritol triacrylate; the infrared spectrum was also consistent with a literature spectrum (*Aldrich*, 1985). The nuclear magnetic resonance spectra indicated a significant number of structurally related impurities in each lot.

The purity of lot 05318JW was determined by Galbraith Laboratories, Inc. (Knoxville, TN), using elemental analyses and by the analytical laboratory using gas chromatography, high-performance liquid chromatography (HPLC), and HPLC with mass spectrometry (HPLC/MS). The purity of lot HCC0340 was determined by the analytical and study laboratories using HPLC. Moisture content was determined by Galbraith Laboratories using Karl Fischer titration.

For lot 05318JW, elemental analyses for carbon, hydrogen, and oxygen were in agreement with the theoretical values for pentaerythritol triacrylate. Karl Fischer titration indicated approximately 943 ppm water. Gas chromatography indicated one major peak, five impurities with areas greater than 1% relative to the major peak area, and six impurities with relative areas between 0.5% and 1%. HPLC indicated a major peak, 18 impurities with areas greater than 1% relative to the major peak area, and five impurities with relative areas between 0.5% and 1%; the concentration of pentaerythritol triacrylate was determined to be approximately 10%. HPLC/MS indicated that eight of the 18 impurity components with relative areas greater than 1% included structurally related adducts, dimers, and acrylates as well as trimethylolpropane triacrylate and its related esters and adducts. No substantial amount of 4-methoxyphenol, a stabilizer added to pentaerythritol triacrylate, was detected. The overall purity of lot 05318JW was determined to be approximately 10% pentaerythritol triacrylate.

For lot HCC0340, HPLC indicated a major peak, seven impurity components with areas greater than 1% of the major peak area, and nine impurity components with relative areas between 0.5% and 1%. By comparison of the retention times of these impurity components to those in the HPLC/MS analysis of lot 05318JW, the impurities were tentatively identified as structurally related adducts, dimers, and acrylates as well as trimethylolpropane triacrylate and its related esters and adducts. The overall purity of lot HCC0340 was determined to be approximately 45% pentaerythritol triacrylate.

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. No degradation of the acetone was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared twice (2-week studies) or approximately every 4 weeks by mixing pentaerythritol triacrylate and acetone to give the required concentration. The dose formulations were stored for up to 35 days for all studies at room temperature in amber glass bottles with Teflon®-lined lids except dose formulations prepared on September 9 and 12, 1996 (3-month studies), which were stored in amber (rat) or clear (mouse) glass vials with Teflon® septa. 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 and a 400 µg/mL formulation 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 pentaerythritol triacrylate were conducted by the study laboratory using gas chromatography. The dose formulations were analyzed once during the 2-week studies; all dose formulations for rats and mice were within 10% of the target concentration. Animal room samples of these dose formulations were also analyzed; all animal room samples for rats and three of five for mice were within 10% of the target concentration. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples of these dose formulations were also analyzed. Of the dose formulations analyzed, all 15 for rats and 14 of 15 for mice were within 10% of the target concentration; 10 of 15 animal room samples for rats and nine of 15 for mice were within 10% of the target concentration. During the 6-month study, the dose formulations were analyzed every 8 or 9 weeks. Of the dose formulations analyzed, 14 of 15 were within 10% of the target concentration; all five animal room samples were also within 10% of the target concentration. The single dose formulation in each of the 3- and 6-month studies that was not within 10% of the target concentration was remixed and reanalyzed and was found to be within 10% of the target concentration. 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 pentaerythritol triacrylate/kg body weight in acetone 5 days per week for 17 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 pentaerythritol 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 12 to 15 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) and 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 27 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 mice were obtained from the NIEHS/NTP colony at Taconic Laboratory Animals and Services. On receipt, the mice were 4 weeks old. Mice 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 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 trimethylolpropane 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, lymph nodes, epididymis, and testis, 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, NTP pathologist, and the 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 Pentaerythritol 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 Service (Germantown, NY)	Taconic Laboratory Animals and Service (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies		
Rats: 11 days Mice: 12 days	Rats: 12 days (male) or 13 days (female) Mice: 14 days (female) or 15 days (male)	11 days
Average Age When Studies Began		
6 weeks	6 weeks	6 weeks
Date of First Dose		
Rats: May 20, 1996 Mice: May 21, 1996	Rats: September 16 (male) or 17 (female), 1996 Mice: September 18 (female) or 19 (male), 1996	July 20, 1998
Duration of Dosing		
5 days per week for 17 days	5 days per week for 14 weeks	Core study: 5 days per week for 27 weeks Positive control: 3 days per week for 28 weeks
Date of Last Dose		
Rats: June 5, 1996 Mice: June 6, 1996	Rats: December 17 (male) or 18 (female), 1996 Mice: December 19 (female) or 20 (male), 1996	Core study: January 19-20 (male) or 20-21 (female), 1999 Positive control: January 27, 1999
Necropsy Dates		
Rats: June 6, 1996 Mice: June 7, 1996	Rats: December 17 (male) or 18 (female), 1996 Mice: December 19 (female) or 20 (male), 1996	Core study: January 19-20 (male) or 20-21 (female), 1999 Positive control: January 27, 1999
Average Age at Necropsy		
8 weeks	19 weeks	32 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

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

2-Week Studies	3-Month Studies	6-Month Study
Animals per Cage		
1	1	1
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 (Lab Products, Inc., Maywood, NJ), 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)

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

2-Week Studies	3-Month Studies	6-Month Study
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 and at the end of the studies 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.
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 Pentaerythritol 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 incidences 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 assumed 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 tumor counts. The two key parameters are γ_1, which measures the dose effect on incidence (proportion 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 (inverse of log) 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 Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of pentaerythritol triacrylate was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and 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.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.,* 1987; Zeiger *et al.,* 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in the acute *in vivo* bone marrow chromosome aberration test or micronucleus test appears to be less than that in the *Salmonella* test (Shelby *et al.,* 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests are associated with 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 males administered 50 mg/kg or greater and 200 mg/kg females were less than those of the vehicle controls. Irritation at the site of application occurred in all dosed groups except 12.5 mg/kg females. Differences in organ weights were not considered biologically significant (Table F1).

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

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	101 ± 3	182 ± 5	81 ± 3	
12.5	5/5	101 ± 2	176 ± 1	76 ± 2	97
25	5/5	100 ± 1	174 ± 2	74 ± 2	96
50	5/5	100 ± 2	171 ± 4*	71 ± 3*	94
100	5/5	100 ± 2	173 ± 2*	73 ± 2*	95
200	5/5	100 ± 2	157 ± 2**	58 ± 3**	86
Female					
0	5/5	85 ± 2	125 ± 2	40 ± 1	
12.5	5/5	84 ± 1	125 ± 2	41 ± 2	100
25	5/5	84 ± 1	124 ± 2	39 ± 1	99
50	5/5	85 ± 2	124 ± 4	39 ± 3	100
100	5/5	84 ± 1	119 ± 1	35 ± 1	95
200	5/5	85 ± 2	114 ± 3**	29 ± 2**	91

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

Crusts at the site of application were observed grossly in all dosed groups except 12.5 mg/kg females. Microscopically, lesions occurred at the site of application in all dosed groups (Table 3). In the 12.5 mg/kg group, epidermal hyperplasia, hyperkeratosis, and sebaceous gland hyperplasia were found in all treated rats and were characterized by increased layers of epidermal cells, thickened keratin layer, and prominence of sebaceous glands. More severe lesions occurred at doses of 25 mg/kg or greater and consisted of ulcer (focal full-thickness necrosis of the epidermis), degeneration (vacuolar change of epidermal cells), parakeratosis (retention of nuclei in keratin layer), mixed inflammatory infiltrate of the dermis (chronic active inflammation), 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 severity of some of the changes (ulcers, degeneration, 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 Pentaerythritol 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** (1.2)[b]	5** (2.2)	5** (2.8)	5** (3.0)	5** (3.0)
Hyperkeratosis	0	5** (1.8)	2 (1.0)	2 (2.0)	1 (2.0)	0
Sebaceous Glands, Hyperplasia	0	5** (1.8)	5** (1.8)	5** (2.6)	4* (2.5)	2 (2.0)
Ulcer	0	0	1 (2.0)	3* (2.3)	4* (3.3)	5** (2.6)
Epidermis, Degeneration	0	0	3* (1.7)	5** (1.6)	2 (1.5)	0
Parakeratosis	0	1 (2.0)	5** (1.8)	5** (2.8)	4* (3.8)	5** (4.0)
Dermis, Inflammation, Chronic Active	0	0	0	1 (2.0)	2 (2.0)	5** (2.4)
Epidermis, Inflammation, Suppurative	0	1 (1.0)	3* (1.7)	5** (2.6)	5** (3.0)	5** (3.6)
Female						
Number Examined						
Microscopically	5	5	5	5	5	5
Epidermis, Hyperplasia	0	5** (1.8)	5** (1.8)	5** (2.6)	5** (2.0)	5** (2.6)
Hyperkeratosis	0	5** (2.0)	3* (2.0)	1 (2.0)	2 (2.0)	1 (1.0)
Sebaceous Glands, Hyperplasia	0	5** (2.2)	5** (1.6)	4* (1.8)	5** (2.0)	4* (1.5)
Ulcer	0	0	0	3* (2.0)	4* (3.5)	4* (3.3)
Epidermis, Degeneration	0	0	1 (2.0)	2 (2.0)	3* (1.3)	2 (1.5)
Parakeratosis	0	0	2 (2.0)	5** (2.8)	4* (3.3)	5** (3.6)
Dermis, Inflammation, Chronic Active	0	0	0	0	5** (2.0)	5** (3.0)
Epidermis, Inflammation, Suppurative	0	0	1 (2.0)	4* (2.5)	5** (3.4)	5** (3.8)

* Significantly different ($P \le 0.05$) from the vehicle control group by the Fisher exact test
** $P \le 0.01$
[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 (Table 4). Final mean body weights and body weight gains of 12 mg/kg males were significantly less than those of the vehicle controls (Table 4 and Figure 1). Irritation at the site of application occurred in 12 mg/kg rats.

The hematology and clinical chemistry data for rats in the 3-month dermal study of pentaerythritol triacrylate are listed in Table E1. On day 23, an increase in segmented neutrophils occurred in 12 mg/kg males and females. At study termination, an increased neutrophil count occurred in 6 mg/kg males. The neutrophilia would be consistent with skin inflammation observed microscopically. No other hematology or clinical chemistry changes were considered to be toxicologically relevant.

Thymus weights of males administered 3 mg/kg or greater were significantly less than those of the vehicle controls (Table F2). There were no significant differences in sperm motility or vaginal cytology parameters between dosed groups and the vehicle controls (Tables G1 and G2).

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

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	86 ± 2	279 ± 11	193 ± 10	
0.75	10/10	85 ± 2	274 ± 7	190 ± 6	98
1.5	10/10	86 ± 2	279 ± 6	193 ± 6	100
3	10/10	85 ± 2	266 ± 7	181 ± 6	95
6	10/10	87 ± 2	265 ± 4	179 ± 4	95
12	10/10	87 ± 3	253 ± 5**	166 ± 5**	91
Female					
0	10/10	86 ± 1	171 ± 4	85 ± 3	
0.75	10/10	86 ± 1	172 ± 3	85 ± 4	100
1.5	10/10	86 ± 1	170 ± 4	84 ± 3	99
3	10/10	86 ± 2	171 ± 3	84 ± 2	100
6	10/10	87 ± 1	168 ± 4	81 ± 4	98
12	10/10	86 ± 2	165 ± 4	79 ± 3	96

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' or Dunnett's test
[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

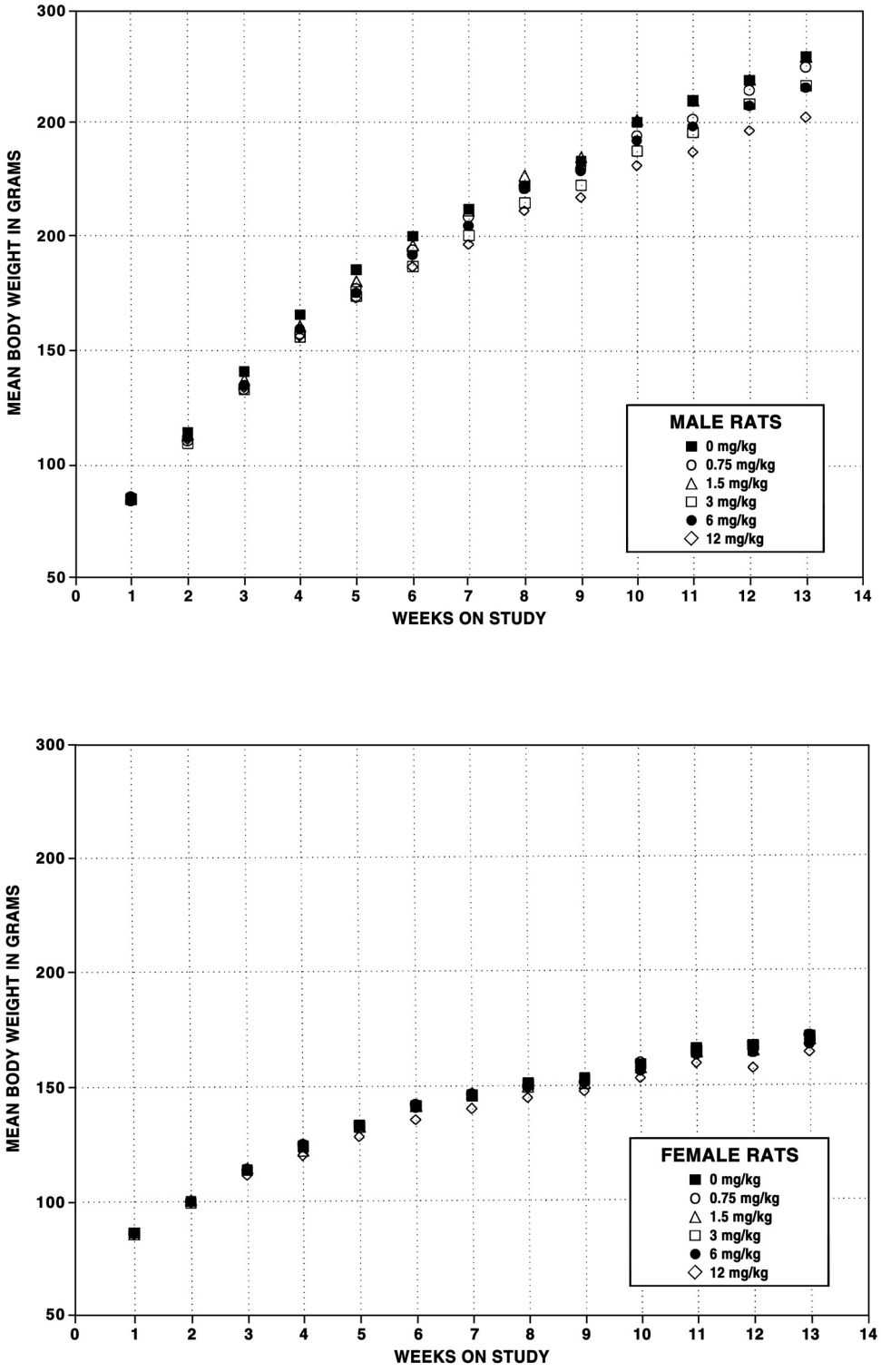


FIGURE 1
Growth Curves for Male and Female Rats
Administered Pentaerythritol Triacrylate Dermally for 3 Months

No treatment-related lesions were observed grossly in rats except irritation at the site of application in the 12 mg/kg groups. Microscopically, the primary changes at the site of application consisted of epidermal hyperplasia, hyperkeratosis, epidermal degeneration and necrosis, and chronic active inflammation (Tables 5, D1, and D2). The incidence and severity of hyperplasia increased with increasing dose; most animals administered 3 mg/kg or greater were affected. Severity was minimal to mild and characterized by focally extensive to diffuse increased thickness of the epidermis, from the normal one to three cell layers thick to four to six layers thick. Hyperplasia was accompanied by minimal to mild increased thickness of the superficial keratin layer (hyperkeratosis). Minimal hyperkeratosis without accompanying hyperplasia was present in the 0.75 and 1.5 mg/kg groups. Degeneration was diagnosed in many animals treated with 1.5 mg/kg or greater. Degeneration was a minimal focal change consisting of intraepidermal vacuolization, presumably due to intra- or intercellular fluid accumulation. Vacuoles occasionally coalesced to form small vesicles that contained a few neutrophils. Epidermal necrosis was present in some males, although a dose-related response was not clear. Necrosis consisted of partial to full-thickness coagulative change of the epidermis and was likely a pathogenic 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 animals administered 1.5 mg/kg or greater, with dose-dependent increases in incidences and severity. Sebaceous glands at the site of application were slightly enlarged and prominent in animals with the other changes described above.

TABLE 5
Incidences of Selected Nonneoplastic Lesions of the Skin (Site of Application) in Rats in the 3-Month Dermal Study of Pentaerythritol 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]	1 (1.0)[b]	2 (1.0)	7** (1.0)	9** (1.0)	10** (1.9)	7** (1.9)
Hyperkeratosis	2 (1.0)	8* (1.0)	10** (1.0)	10** (1.0)	10** (1.7)	9** (1.3)
Epidermis, Degeneration	0	1 (1.0)	6** (1.0)	7** (1.0)	7** (1.3)	5* (1.2)
Epidermis, Necrosis	0	0	1 (1.0)	1 (1.0)	5* (1.2)	2 (1.0)
Epidermis, Inflammation, Suppurative	0	0	0	0	6** (1.2)	3 (1.0)
Dermis, Inflammation, Chronic Active	0	1 (1.0)	3 (1.0)	10** (1.0)	10** (1.5)	9** (1.4)
Sebaceous Gland, Hyperplasia	0	2 (1.0)	9** (1.0)	9** (1.1)	10** (1.1)	9** (1.8)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Epidermis, Hyperplasia	0	0	3 (1.0)	8** (1.1)	8** (1.9)	9** (2.0)
Hyperkeratosis	0	4* (1.0)	8** (1.1)	9** (1.2)	10** (1.9)	10** (1.4)
Epidermis, Degeneration	0	0	4* (1.0)	5* (1.0)	5* (1.2)	6** (1.2)
Epidermis, Inflammation, Suppurative	0	0	0	0	4* (1.3)	0
Dermis, Inflammation, Chronic Active	0	0	3 (1.0)	4* (1.0)	9** (1.3)	10** (1.4)
Sebaceous Gland, Hyperplasia	0	0	8** (1.0)	8** (1.0)	10** (1.6)	9** (1.6)

* 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 and body weight gain of 25 mg/kg males were significantly greater than those of the vehicle controls, as was the mean body weight gain of 50 mg/kg males. Irritation at the site of application occurred in all dosed groups. Thymus weights of 50 mg/kg or greater males and of 200 mg/kg females were significantly less than those of the vehicle controls (Table F3).

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

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	22.2 ± 0.4	24.6 ± 0.4	2.4 ± 0.1	
12.5	5/5	22.1 ± 0.2	25.3 ± 0.3	3.2 ± 0.3	103
25	5/5	21.9 ± 0.2	26.1 ± 0.2**	4.2 ± 0.1**	106
50	5/5	21.2 ± 0.5	25.4 ± 0.4	4.2 ± 0.3**	103
100	5/5	21.7 ± 0.6	24.3 ± 0.3	2.6 ± 0.6	99
200	5/5	21.4 ± 0.5	24.2 ± 0.3	2.8 ± 0.6	98
Female					
0	5/5	17.3 ± 0.3	20.9 ± 0.6	3.6 ± 0.6	
12.5	5/5	16.7 ± 0.5	21.2 ± 0.4	4.4 ± 0.6	101
25	5/5	16.9 ± 0.5	21.9 ± 0.7	4.9 ± 0.8	105
50	5/5	16.9 ± 0.7	19.6 ± 1.3	2.7 ± 1.3	93
100	5/5	17.6 ± 0.7	22.1 ± 0.6	4.5 ± 0.3	106
200	5/5	17.5 ± 0.8	21.2 ± 0.5	3.7 ± 0.3	101

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' or Dunnett's test
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

Crusts were observed grossly at the site of application in all dosed groups. Microscopically, lesions were found at the site of application in all dosed groups (Table 7). Epidermal hyperplasia, hyperkeratosis, and sebaceous gland hyperplasia were found in most 12.5 mg/kg mice. These lesions were characterized by increased layers of epidermal cells, a thickened keratin layer, and prominence of sebaceous glands. More severe lesions that tended to occur at higher doses were ulcer (focal full-thickness necrosis of the epidermis), degeneration (vacuolar change of epidermal cells), parakeratosis (retention of nuclei in the keratin layer), dermal chronic active inflammation (mixed inflammatory cell infiltrate), and epidermal suppurative inflammation (accumulation of neutrophils) 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 skin lesions, particularly ulcer, degeneration, and/or suppurative inflammation, that occurred in dosed mice precluded the use of doses of 25 mg/kg or greater in the 3-month study. Therefore, the doses selected for the 3-month study in $B6C3F_1$ mice were 0, 0.75, 1.5, 3, 6, and 12 mg/kg.

TABLE 7
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in $B6C3F_1$ Mice in the 2-Week Dermal Study of Pentaerythritol 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** (1.6)[b]	5** (1.8)	5** (2.0)	5** (3.4)	5** (3.8)
Hyperkeratosis	0	2 (1.5)	5** (1.2)	5** (1.2)	3* (2.0)	0
Sebaceous Glands, Hyperplasia	0	5** (3.0)	5** (2.2)	3* (1.3)	1 (1.0)	0
Ulcer	0	0	0	0	5** (2.6)	5** (3.2)
Epidermis, Degeneration	0	0	3* (1.0)	1 (1.0)	1 (1.0)	4* (1.3)
Parakeratosis	0	4* (1.0)	5** (2.0)	2 (1.5)	2 (2.0)	4* (3.8)
Dermis, Inflammation, Chronic Active	0	0	4* (1.0)	2 (1.5)	3* (2.0)	5** (2.4)
Epidermis, Inflammation, Suppurative	0	0	3* (1.0)	4* (1.0)	5** (2.4)	5** (3.4)
Female						
Number Examined						
Microscopically	5	5	5	5	5	5
Epidermis, Hyperplasia	0	5** (1.8)	5** (1.0)	5** (2.6)	5** (3.4)	5** (3.6)
Hyperkeratosis	0	5** (1.4)	3* (1.0)	1 (2.0)	3 (2.3)	0
Sebaceous Glands, Hyperplasia	0	5** (2.8)	5** (2.6)	5** (2.6)	3* (3.0)	1 (3.0)
Ulcer	0	0	0	1 (1.0)	1 (3.0)	5** (2.4)
Epidermis, Degeneration	0	0	0	1 (1.0)	1 (1.0)	3* (1.0)
Parakeratosis	0	0	2 (1.5)	3* (2.0)	4* (1.5)	5** (3.2)
Dermis, Inflammation, Chronic Active	0	0	1 (1.0)	2 (1.5)	3* (2.3)	4* (2.5)
Epidermis, Inflammation, Suppurative	0	0	1 (1.0)	3* (1.3)	5** (2.2)	4* (3.0)

* 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 lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

3-Month Study in B6C3F$_1$ Mice

One female vehicle control mouse was sacrificed during the first week of the study due to ataxia and one 1.5 mg/kg female died during week 8; all other mice survived to the end of the study (Table 8). There were no significant differences in final mean body weights or body weight gains between the dosed and vehicle control groups (Table 8 and Figure 2). Irritation at the site of application occurred in the 6 and 12 mg/kg male groups.

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

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	21.7 ± 0.3	32.5 ± 0.5	10.8 ± 0.5	
0.75	10/10	21.9 ± 0.5	34.9 ± 0.8	13.0 ± 0.6	107
1.5	10/10	21.6 ± 0.5	34.0 ± 0.7	12.4 ± 0.8	105
3	10/10	20.6 ± 0.8	33.0 ± 1.0	12.4 ± 1.2	102
6	10/10	21.5 ± 0.3	31.8 ± 0.3	10.4 ± 0.3	98
12	10/10	21.6 ± 0.6	32.8 ± 0.5	11.2 ± 0.6	101
Female					
0	9/10[c]	19.0 ± 0.5	30.8 ± 0.7	11.5 ± 0.5	
0.75	10/10[d]	19.1 ± 0.4	30.1 ± 1.0	11.1 ± 0.8	98
1.5	9/10[d]	19.4 ± 0.6	29.7 ± 0.8	10.0 ± 0.5	97
3	10/10	19.6 ± 0.4	30.4 ± 0.8	10.8 ± 0.7	99
6	10/10	19.6 ± 0.3	29.5 ± 0.8	9.9 ± 0.6	96
12	10/10	18.9 ± 0.3	28.5 ± 0.4	9.7 ± 0.3	93

[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Week of death: 1
[d] Week of death: 8

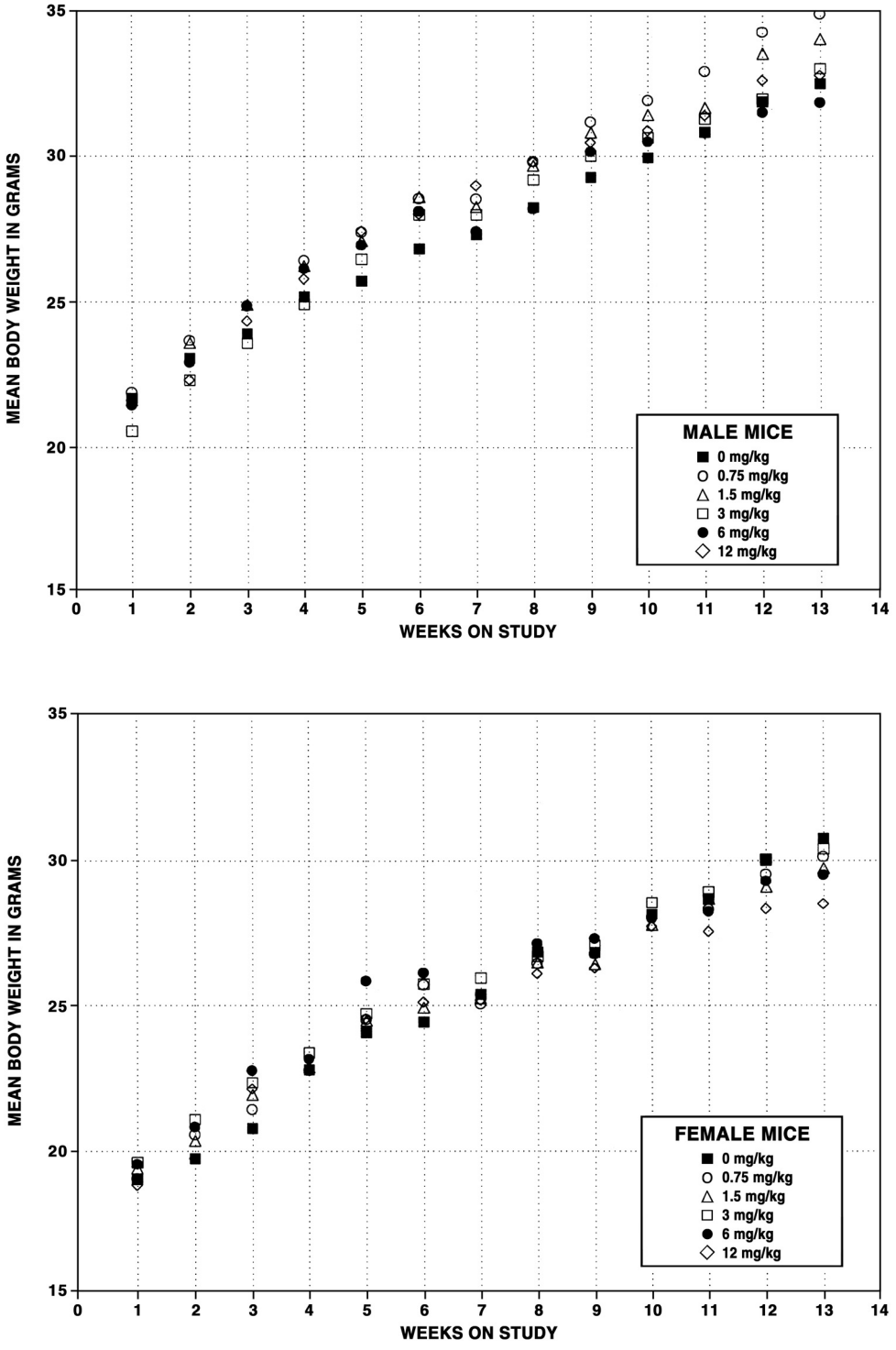


FIGURE 2
Growth Curves for Male and Female B6C3F$_1$ Mice Administered Pentaerythritol Triacrylate Dermally for 3 Months

The hematology data for mice in the 3-month dermal study of pentaerythritol triacrylate are listed in Table E2. Similar to the rat study, an increase in segmented neutrophils occurred in the 6 mg/kg male mice and 12 mg/kg male and female mice and would be consistent with the skin inflammation observed microscopically. A minimal decrease (approximately 6% or less) in the hematocrit, hemoglobin concentration, and erythrocyte count occurred in 6 and 12 mg/kg mice and would be consistent with minimal erythropoietic suppression related to inflammation.

There were no biologically significant differences in organ weights between dosed and vehicle control groups (Table F4). The number of spermatid heads per testis in 3 mg/kg males and the numbers of spermatid heads per gram cauda in 6 and 12 mg/kg males were significantly greater than those in the vehicle controls (Table G3); however, these differences were not considered to be biologically significant. There were no significant differences in vaginal cytology parameters between the dosed and vehicle control groups (Table G4).

No lesions were observed grossly in mice except irritation at the site of application in 6 and 12 mg/kg males. Similar to the effects in rats, the primary microscopic changes at the site of application were epidermal hyperplasia, degeneration, and necrosis; dermal chronic active inflammation, and sebaceous gland hyperplasia (Tables 9, D3, and D4). The incidences of epidermal hyperplasia increased with increasing dose, and the severity was generally greater in the 6 and 12 mg/kg groups; most mice administered 3 mg/kg or greater were affected, with lower incidences in lower dose groups. Severity was minimal to moderate and characterized by focally extensive to diffuse increased thickness of the epidermis, from the normal one to three cell layers thick to four to eight layers thickness. Hyperplasia was accompanied by minimal to mild increased thickness of the superficial keratin layer (hyperkeratosis). Degeneration was diagnosed in several male mice exposed to 1.5 mg/kg or greater, but there was no clear dose-related response trend. Degeneration was a minimal, focal change consisting of intraepidermal vacuolization, presumably due to intra- or intercellular fluid accumulation. Epidermal necrosis was diagnosed with increasing incidence and severity in male mice at doses of 1.5 mg/kg and greater. 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 the dermis of most animals administered 1.5 mg/kg or greater, with dose-dependent increases in incidences and severity. Slight superficial dermal fibrosis occurred in some 6 and 12 mg/kg mice. Sebaceous glands at the site of application were slightly enlarged and prominent in mice with the other changes described above, an effect that was likely the result of dermal irritation.

Dose Selection Rationale: There were no effects on survival or body weight of $B6C3F_1$ mice treated with pentaerythritol triacrylate in the 3-month study. In selecting doses for a 2-year dermal administration bioassay based on findings in 3-months studies, dose levels containing any severity of ulceration and necrosis or other changes (e.g. inflammation or hyperplasia) at the skin site of application that are moderate to marked are generally avoided. Therefore, because of the presence of some of these lesions, 6 and 12 mg/kg would not have been selected for use in a 2-year bioassay for the males. The severity of lesions likely would have precluded use of 12 mg/kg in females. The incidence and/or severity of lesions was generally lower in females administered 6 mg/kg or less and males administered 3 mg/kg or less. Because doses were being selected for a different strain (Tg.AC) and for a different duration (6 months), five dose groups were used to allow a margin of error, ranging from doses that produced minimal or 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 Pentaerythritol 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]	8** (1.1)	9** (1.9)	10** (1.6)
Epidermis, Degeneration	0	0	5* (1.0)	5* (1.4)	2 (1.0)	4* (1.0)
Epidermis, Necrosis	0	0	2 (1.5)	3 (1.3)	7** (1.4)	7** (2.4)
Dermis, Inflammation, Chronic Active	0	0	8** (1.0)	8** (1.0)	10** (1.6)	10** (1.4)
Sebaceous Gland, Hyperplasia	0	0	7** (1.0)	9** (1.7)	10** (2.1)	10** (1.7)
Hyperkeratosis	0	0	5* (1.0)	7** (1.3)	8** (1.5)	6** (1.2)
Dermis, Fibrosis	0	0	0	0	6** (1.2)	10** (1.7)
Female						
Number Examined Microscopically	9	10	10	10	10	10
Epidermis, Hyperplasia	0	1 (1.0)	2 (1.0)	6** (1.3)	8** (1.6)	10** (1.3)
Dermis, Inflammation, Chronic Active	0	2 (1.0)	5* (1.0)	9** (1.0)	9** (1.4)	10** (2.1)
Sebaceous Gland, Hyperplasia	0	0	9** (1.0)	9** (1.3)	8** (1.6)	10** (2.2)
Hyperkeratosis	0	1 (1.0)	2 (1.0)	7** (1.1)	7** (1.3)	7** (1.3)
Dermis, Fibrosis	0	0	0	0	9** (1.3)	10** (2.2)

* 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

6-Month Study in Tg.AC Hemizygous Mice

Estimates of 6-month survival probabilities for male and female Tg.AC hemizygous 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 Pentaerythritol 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
Moribund	0	1	0	0	1	0
Natural deaths	3	0	0	0	2	5
Animals surviving to study termination	12	14	15	15	12	10
Percent probability of survival at end of study[a]	80	93	100	100	80	67
Mean survival (days)[b]	177	181	185	185	182	178
Survival analysis[c]	P=0.031	P=0.616N	P=0.224N	P=0.224N	P=1.000N	P=0.762
Female						
Animals initially in study	15	15	15	15	15	15
Moribund	1	1	0	0	0	3
Natural deaths	2	0	3	3	2	3
Animals surviving to study termination	12	14	12	12	13	9
Percent probability of survival at end of study	80	93	80	80	87	60
Mean survival (days)	170	178	166	184	179	156
Survival analysis	P=0.087	P=0.592N	P=1.000	P=1.000N	P=0.922N	P=0.416

[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**.

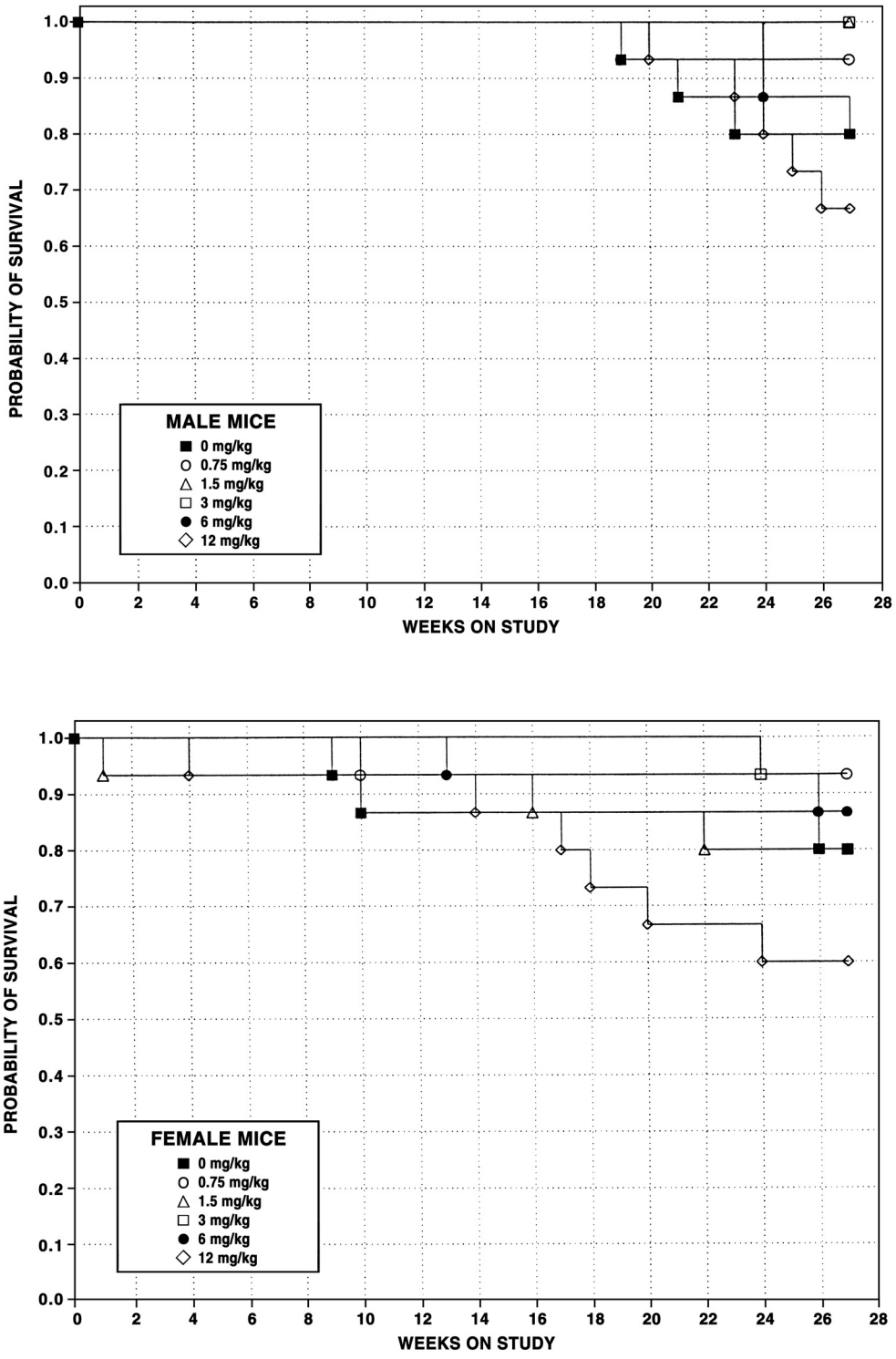


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Tg.AC Hemizygous Mice Administered Pentaerythritol Triacrylate Dermally for 6 Months

Body Weights and Clinical Findings

With the exception of the 3 mg/kg group, body weights of male mice were less than those of the vehicle controls during the last 3 to 6 weeks of the study (Figure 4 and Table 11). Females administered 3 mg/kg had generally reduced body weights during the last month of the study (Figure 4 and Table 12). Treatment-related clinical findings included masses on the torso of males and females administered 3 mg/kg or greater; a single 0.75 mg/kg male and two 1.5 mg/kg males were also affected. Papilloma occurred at the site of application in the 3 mg/kg or greater male and female groups (Table 13). Papillomas were observed earlier in the 6 and 12 mg/kg groups than in the 3 mg/kg groups. One vehicle control male, two 1.5 mg/kg males, and one 1.5 mg/kg female also had in-life papillomas.

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

Heart and liver weights of 12 mg/kg males were significantly greater than those of the vehicle controls (Table F5). Lung weights of 6 and 12 mg/kg males and females were significantly decreased, as were thymus weights of 6 and 12 mg/kg females.

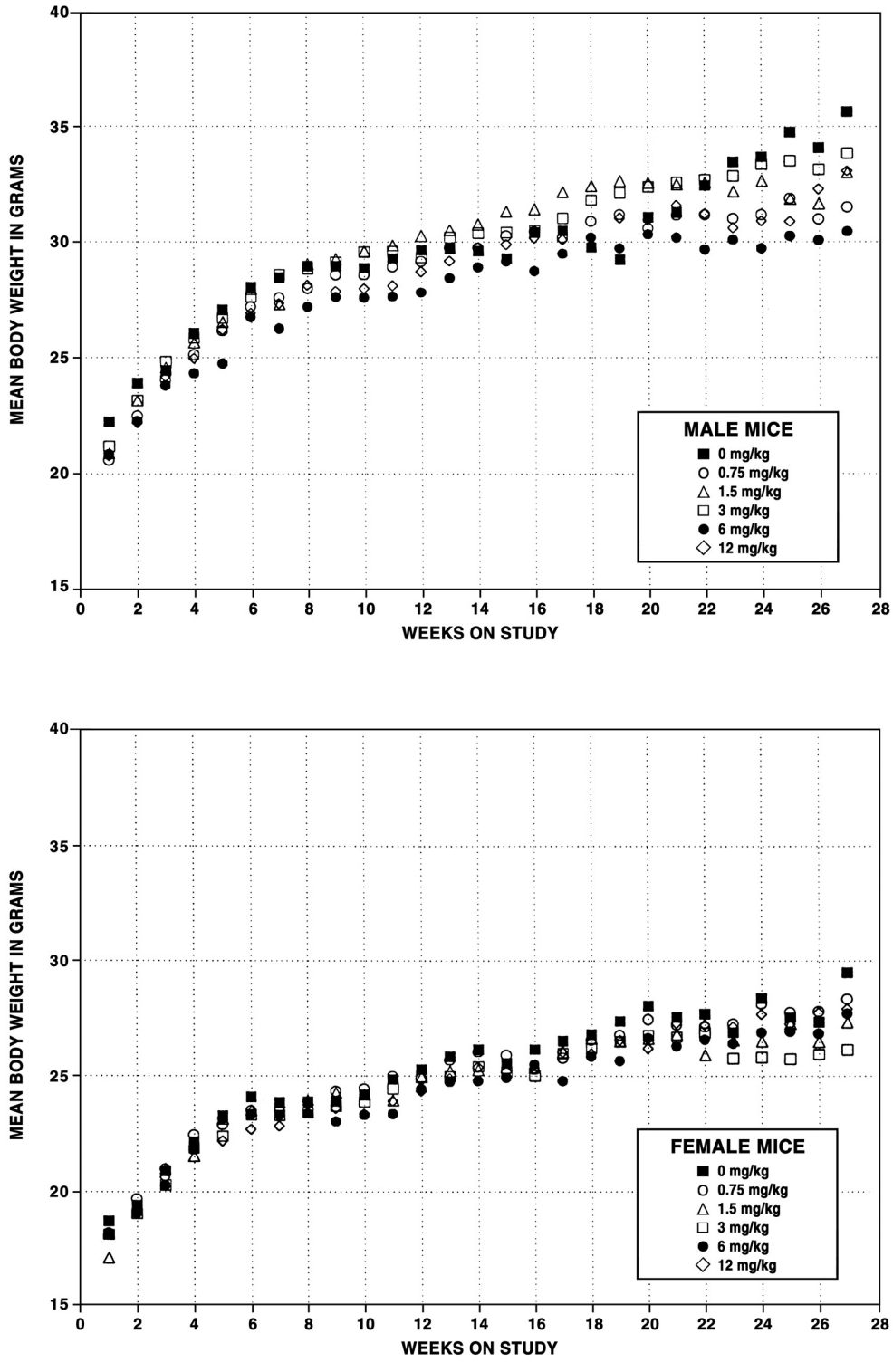


FIGURE 4
Growth Curves for Male and Female Tg.AC Hemizygous Mice Administered Pentaerythritol Triacrylate Dermally for 6 Months

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

Weeks on Study	Vehicle Control		0.75 mg/kg			1.5 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
1	22.3	15	20.6	92	15	20.9	94	15
2	23.9	15	22.5	94	15	23.2	97	15
3	24.5	15	24.1	98	15	24.6	100	15
4	26.1	15	25.1	96	15	25.7	99	15
5	27.1	15	26.2	97	15	26.5	98	15
6	28.0	15	27.2	97	15	28.0	100	15
7	28.5	15	27.6	97	15	27.3	96	15
8	29.0	15	28.0	97	15	29.1	100	15
9	29.0	15	28.6	99	15	29.3	101	15
10	28.9	15	28.6	99	15	29.6	102	15
11	29.3	15	28.9	99	15	29.9	102	15
12	29.7	15	29.2	98	15	30.3	102	15
13	29.7	15	29.8	100	15	30.5	103	15
14	29.6	15	29.8	101	15	30.8	104	15
15	29.3	15	30.3	103	15	31.3	107	15
16	30.4	15	30.5	100	15	31.4	103	15
17	30.5	15	30.2	99	15	32.2	106	15
18	29.8	15	30.9	104	15	32.4	109	15
19	29.2	15	31.2	107	15	32.7	112	15
20	31.1	14	30.6	98	14	32.6	105	15
21	31.3	14	31.2	100	14	32.5	104	15
22	32.5	13	31.2	96	14	32.6	100	15
23	33.5	12	31.0	93	14	32.2	96	15
24	33.7	12	31.2	93	14	32.7	97	15
25	34.8	12	31.9	92	14	31.9	92	15
26	34.1	12	31.0	91	14	31.7	93	15
27	35.7	12	31.5	88	14	33.0	92	15
Mean for weeks								
1-13	27.4		26.6	97		27.3	100	
14-27	31.8		30.9	97		32.1	101	

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

Weeks on Study	3 mg/kg			6 mg/kg			12 mg/kg		
	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	21.2	95	15	20.9	94	15	20.8	93	15
2	23.2	97	15	22.3	93	15	22.2	93	15
3	24.8	101	15	23.8	97	15	24.2	99	15
4	25.9	99	15	24.3	93	15	25.0	96	15
5	26.7	99	15	24.8	92	15	26.2	97	15
6	27.6	99	15	26.8	96	15	26.9	96	15
7	28.6	100	15	26.3	92	15	27.3	96	15
8	28.8	99	15	27.2	94	15	28.1	97	15
9	29.1	100	15	27.6	95	15	27.9	96	15
10	29.6	102	15	27.6	96	15	28.0	97	15
11	29.6	101	15	27.7	95	15	28.1	96	15
12	29.3	99	15	27.8	94	15	28.7	97	15
13	30.2	102	15	28.5	96	15	29.2	98	15
14	30.4	103	15	28.9	98	15	29.8	101	15
15	30.4	104	15	29.2	100	15	29.9	102	15
16	30.5	100	15	28.8	95	15	30.2	99	15
17	31.0	102	15	29.5	97	15	30.1	99	15
18	31.8	107	15	30.2	101	15	30.2	101	15
19	32.1	110	15	29.7	102	15	31.1	107	15
20	32.4	104	15	30.4	98	15	31.0	100	15
21	32.6	104	15	30.2	97	15	31.6	101	14
22	32.7	101	15	29.7	91	15	31.2	96	14
23	32.9	98	15	30.1	90	15	30.6	91	14
24	33.4	99	15	29.7	88	15	30.9	92	13
25	33.5	96	15	30.3	87	13	30.9	89	12
26	33.1	97	15	30.1	88	13	32.3	95	11
27	33.9	95	15	30.5	85	13	33.1	93	10
Mean for weeks									
1-13	27.3	100		25.8	94		26.4	96	
14-27	32.2	101		29.8	94		30.9	97	

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

Weeks on Study	Vehicle Control		0.75 mg/kg			1.5 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
1	18.7	15	18.2	97	15	17.2	92	15
2	19.4	15	19.7	102	15	19.3	100	14
3	20.9	15	21.0	101	15	21.0	101	14
4	22.2	15	22.5	101	15	21.6	97	14
5	23.3	15	22.9	98	15	23.2	100	14
6	24.1	15	23.5	98	15	23.5	98	14
7	23.9	15	23.5	98	15	23.9	100	14
8	23.9	15	23.9	100	15	24.0	100	14
9	23.9	15	24.4	102	15	24.3	102	14
10	24.2	14	24.5	101	15	24.2	100	14
11	24.9	13	25.0	100	14	24.0	96	14
12	25.3	13	24.9	98	14	25.0	99	14
13	25.8	13	25.7	100	14	25.2	98	14
14	26.1	13	26.1	100	14	25.3	97	14
15	25.6	13	25.9	101	14	25.4	99	14
16	26.2	13	25.3	97	14	25.4	97	14
17	26.5	13	25.8	97	14	26.0	98	13
18	26.8	13	26.6	99	14	26.6	99	13
19	27.4	13	26.8	98	14	26.5	97	13
20	28.0	13	27.5	98	14	26.6	95	13
21	27.6	13	27.2	99	14	26.8	97	13
22	27.7	13	27.1	98	14	25.9	94	13
23	26.9	13	27.3	102	14	26.6	99	12
24	28.4	13	28.1	99	14	26.5	93	12
25	27.5	13	27.8	101	14	27.3	99	12
26	27.4	13	27.8	102	14	26.5	97	12
27	29.5	12	28.4	96	14	27.3	93	12
Mean for weeks								
1-13	23.1		23.1	100		22.8	99	
14-27	27.3		27.0	99		26.3	96	

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

Weeks on Study	3 mg/kg			6 mg/kg			12 mg/kg		
	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	18.2	97	15	18.3	98	15	18.2	97	15
2	19.1	99	15	19.1	99	15	19.2	99	15
3	20.3	97	15	20.3	97	15	20.6	99	15
4	21.9	99	15	21.8	98	15	22.0	99	15
5	22.4	96	15	23.2	100	15	22.2	95	14
6	23.3	97	15	23.4	97	15	22.7	94	14
7	23.3	98	15	23.3	98	15	22.9	96	14
8	23.4	98	15	23.4	98	15	23.4	98	14
9	23.7	99	15	23.1	97	15	23.6	99	14
10	23.9	99	15	23.3	96	15	23.4	97	14
11	24.5	98	15	23.4	94	15	23.9	96	14
12	25.0	99	15	24.5	97	15	24.4	96	14
13	25.0	97	15	24.8	96	14	24.9	97	14
14	25.4	97	15	24.8	95	14	25.4	97	14
15	25.2	98	15	24.9	97	14	25.2	98	13
16	25.0	95	15	25.5	97	14	25.3	97	13
17	26.0	98	15	24.8	94	14	26.1	99	12
18	26.2	98	15	25.9	97	14	26.0	97	12
19	26.5	97	15	25.7	94	14	26.6	97	11
20	26.8	96	15	26.7	95	14	26.2	94	11
21	26.7	97	15	26.3	95	14	27.3	99	10
22	26.9	97	15	26.6	96	14	27.2	98	10
23	25.8	96	15	26.4	98	14	27.1	101	10
24	25.8	91	15	26.9	95	14	27.7	98	9
25	25.8	94	14	26.9	98	14	27.3	99	9
26	26.0	95	14	26.9	98	14	27.7	101	9
27	26.1	89	12	27.7	94	13	27.9	95	9
Mean for weeks									
1-13	22.6	98		22.5	97		22.4	97	
14-27	26.0	95		26.1	96		26.6	97	

TABLE 13
Skin Papilloma Formation at the Site of Application in Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol 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	1	(7.4%)	27	>27	0	0	0		
0.75	0	(0.0%)	NA	>27	0	0	0		NT
1.5	2	(13.3%)	20	>27	0	0	0		NT
3	13	(86.7%)	14	20	1	3	9	**	NT
6	15	(100.0%)	9	13	20+	20+	20+	**	NT
12	13	(86.7%)	9	10	20+	20+	20+	**	NT
Trend								**	**
Positive Control[f]	15	(100.0%)	9	10	9	20+	20+	**	NT
Female									
0	0	(0.0%)	NA	>27	0	0	0		
0.75	0	(0.0%)	NA	>27	0	0	0		NT
1.5	1	(7.8%)	27	>27	0	0	0		NT
3	10	(68.5%)	17	24	0	1	4	**	NT
6	13	(86.7%)	11	13	10	20+	20+	**	NT
12	13	(92.8%)	9	11	15	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] If the first papilloma was observed at the terminal sacrifice, it was assigned a time to first occurrence of 27 weeks. For groups in which fewer than half of the animals had papillomas, the median time to initial occurrence is >27 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 these data do not support a pairwise test. ** ($P \leq 0.01$) 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, liver, spleen, mandibular lymph node, kidney, thymus, and epididymis. 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: Histologic evaluation revealed that squamous cell papillomas at the site of application were present at the end of the study in all 3 and 6 mg/kg males, most females administered 3 or 6 mg/kg, and most 12 mg/kg males and females (Tables 14, A3, and B3). Two 3 mg/kg and three 12 mg/kg males and one 12 mg/kg female had squamous cell carcinomas at the site of application. Significantly increased incidences of nonneoplastic lesions occurred at the site of application, including hyperkeratosis in 3 mg/kg or greater males and females, chronic active inflammation in 6 and 12 mg/kg males and females and 3 mg/kg females, and epidermal hyperplasia in males and females administered 3 mg/kg or greater; the incidence of epidermal hyperplasia was also significantly increased in 1.5 mg/kg females. The severity of epidermal hyperplasia increased with increasing dose.

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 Pentaerythritol 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
Hyperkeratosis[a]	1 (2.0)[b]	0	2 (2.0)	6* (1.0)	10** (1.8)	13** (1.5)
Inflammation, Chronic Active	0	0	0	2 (1.0)	5* (1.2)	12** (1.3)
Epidermis, Hyperplasia	0	0	3 (1.0)	8** (1.8)	10** (1.9)	14** (2.4)
Squamous Cell Papilloma, Multiple	0	0	0	11**	15**	13**
Squamous Cell Papilloma (includes multiple)						
Overall rate[c]	1/15 (7%)	0/15 (0%)	4/15 (27%)	15/15 (100%)	15/15 (100%)	13/15 (87%)
Adjusted rate[d]	7.4%	0.0%	26.7%	100.0%	100.0%	86.7%
Terminal rate[e]	1/12 (8%)	0/14 (0%)	4/15 (27%)	15/15 (100%)	12/12 (100%)	8/10 (80%)
First incidence (days)	185 (T)	—[g]	185 (T)	185 (T)	163	138
Poly-3 test[f]	P<0.001	P=0.487N	P=0.198	P<0.001	P<0.001	P<0.001
Squamous Cell Carcinoma, Multiple	0	0	0	0	0	1
Squamous Cell Carcinoma (includes multiple)	0	0	0	2	0	3
Squamous Cell Papilloma or Squamous Cell Carcinoma						
Overall rate	1/15 (7%)	0/15 (0%)	4/15 (27%)	15/15 (100%)	15/15 (100%)	13/15 (87%)
Adjusted rate	7.4%	0.0%	26.7%	100.0%	100.0%	86.7%
Terminal rate	1/12 (8%)	0/14 (0%)	4/15 (27%)	15/15 (100%)	12/12 (100%)	8/10 (80%)
First incidence (days)	185 (T)	—	185 (T)	185 (T)	163	138
Poly-3 test	P<0.001	P=0.487N	P=0.198	P<0.001	P<0.001	P<0.001

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 Pentaerythritol Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Female						
Number Examined						
Microscopically	15	15	15	15	15	15
Hyperkeratosis	1 (2.0)	0	4 (1.5)	14** (1.6)	11** (1.5)	13** (2.0)
Inflammation, Chronic Active	0	0	1 (1.0)	9** (1.1)	10** (1.7)	15** (1.5)
Epidermis, Hyperplasia	0	0	5* (1.2)	14** (2.0)	14** (2.4)	14** (2.9)
Squamous Cell Papilloma, Multiple	0	0	1	7**	12**	13**
Squamous Cell Papilloma (includes multiple)						
Overall rate	0/15 (0%)	0/15 (0%)	1/15 (7%)	10/15 (67%)	12/15 (80%)	13/15 (87%)
Adjusted rate	0.0%	0.0%	7.9%	68.4%	85.1%	92.8%
Terminal rate	0/12 (0%)	0/14 (0%)	1/12 (8%)	9/12 (75%)	11/13 (85%)	8/9 (89%)
First incidence (days)	—	—[h]	186 (T)	177	182	98
Poly-3 test	P<0.001	—	P=0.496	P<0.001	P<0.001	P<0.001
Squamous Cell Carcinoma	0	0	0	0	0	1
Squamous Cell Papilloma or Squamous Cell Carcinoma						
Overall rate	0/15 (0%)	0/15 (0%)	1/15 (7%)	10/15 (67%)	12/15 (80%)	13/15 (87%)
Adjusted rate	0.0%	0.0%	7.9%	68.4%	85.1%	92.8%
Terminal rate	0/12 (0%)	0/14 (0%)	1/12 (8%)	9/12 (75%)	11/13 (85%)	8/9 (89%)
First incidence (days)	—	—	186 (T)	177	182	98
Poly-3 test	P<0.001	—	P=0.496	P<0.001	P<0.001	P<0.001

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
(T) Terminal sacrifice
[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. A lower incidence in a dosed group is indicated by **N**.
[g] Not applicable; no neoplasms in animal group
[h] Value of statistic cannot be computed.

Other Organs: Males administered 0.75, 3, or 6 mg/kg had significantly increased incidences of chronic active inflammation of the liver (Table 15). The incidences of hematopoietic cell proliferation of the liver in 12 mg/kg females and of the spleen in 6 and 12 mg/kg males and females were significantly increased. Females administered 12 mg/kg had significantly increased incidences of hematopoietic cell proliferation of the mandibular lymph node and thymocyte necrosis.

There was a change that occurred in several 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 five 12 mg/kg males, while milder lesions, diagnosed as cell, infiltration, nonspecified site, were identified in several animals. 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 15
Incidences of Selected Nonneoplastic Lesions in Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol 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	
Inflammation, Chronic Active[b]	5	(1.0)[c]	11*	(1.0)	10	(1.0)	13**	(1.0)	13**	(1.1)	5	(1.0)
Spleen	15		15		15		15		15		15	
Hematopoietic Cell Proliferation	1	(2.0)	2	(2.0)	1	(2.0)	2	(2.0)	9**	(2.1)	8**	(2.5)
All Organs[d]	15		15		15		15		15		15	
Myelodysplasia	0		0		0		0		0		5*	
Infiltration Cellular	0		0		0		0		6**		6**	
Infiltration Cellular, Plasma Cell	0		0		0		0		3		6**	
Female												
Liver	15		15		15		15		15		15	
Hematopoietic Cell Proliferation	0		0		0		1	(1.0)	1	(2.0)	6**	(1.5)
Spleen	15		15		15		15		15		15	
Hematopoietic Cell Proliferation	0		0		0		2	(2.0)	11**	(2.2)	11**	(2.5)
Lymph Node, Mandibular	15		15		15		15		15		13	
Hematopoietic Cell Proliferation	0		0		0		0		1	(2.0)	8**	(1.8)
Thymus	15		15		15		14		14		15	
Thymocyte, Necrosis	0		1	(4.0)	2	(3.5)	2	(2.0)	0		5*	(2.4)
All Organs	15		15		15		15		15		15	
Infiltration Cellular	0		0		0		1		4*		2	
Infiltration Cellular, Plasma Cell	0		0		0		0		1		1	

* 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 pentaerythritol triacrylate treatment in dosed mice. Results of the irritancy studies of the approximately 10% and 45% pentaerythritol triacrylate mixtures indicated that the maximal nonirritating and minimal irritating doses were 0.1% and 0.25% pentaerythritol triacrylate, respectively, for each mixture (Figures K4 and K5).

The mouse ear swelling test did not indicate the approximately 10% pentaerythritol triacrylate mixture as a contact sensitizer in female BALB/c mice at the doses tested 24 or 48 hours after dosing (Figure K6). Testing of the approximately 45% pentaerythritol triacrylate mixture with the ear swelling test paradigm indicated pentaerythritol triacrylate as a contact sensitizer at 0.1%, with significant increases (compared to background controls) in percent ear swelling noted 24 and 48 hours postchallenge (Figure K7). In the first local lymph node assay using the approximately 10% pentaerythritol triacrylate mixture, a significant increase in lymph node cell proliferation occurred in all dosed groups of mice compared to the vehicle controls; in the second assay using lower sensitizing doses, a significant response occurred only in the 0.05% group (Figure K8). Local lymph node assay of the approximately 45% pentaerythritol triacrylate mixture yielded a positive response only with a sensitizing concentration of 0.25% (Figure K9).

In summary, for both the approximately 10% and 45% pentaerythritol triacrylate mixtures, the maximal nonirritating concentration was determined to be 0.1%, and the minimal irritating concentration to be 0.25%. The mouse ear swelling test yielded negative results for pentaerythritol triacrylate as a potential contact sensitizer when the approximately 10% pentaerythritol triacrylate mixture was used and positive results when the approximately 45% pentaerythritol triacrylate mixture was used. Positive responses were seen in local lymph node assays at concentrations of 0.05%, 0.1%, and 0.25% pentaerythritol triacrylate when the approximately 10% pentaerythritol triacrylate mixture was used and at a concentration of 0.25% pentaerythritol triacrylate when the approximately 45% pentaerythritol triacrylate mixture was used. These data indicate the potential of pentaerythritol triacrylate as a weak contact sensitizer.

Genetic Toxicology

Pentaerythritol triacrylate (33 to 10,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537 with or without Aroclor-induced rat or hamster S9 enzymes (Zeiger *et al.*, 1987; Table C1). No increase in the frequency of micronucleated normochromatic erythrocytes (NCEs) was observed in peripheral blood samples from male or female $B6C3F_1$ mice treated dermally with pentaerythritol triacrylate for 3 months. In contrast, treatment of Tg.AC hemizygous female mice administered pentaerythritol triacrylate dermally for 6 months induced a significant increase in micronucleated NCEs; the trend test analysis was highly significant ($P \leq 0.001$), and the two highest dose groups showed mean values significantly elevated over the vehicle control frequency. In Tg.AC hemizygous male mice treated with pentaerythritol triacrylate for 6 months, a small increase in micronucleated NCEs was detected; however, the response was judged to be equivocal due to a positive trend test ($P=0.001$) without any one dose group being significantly elevated over the vehicle control frequency. In both micronucleus studies, the dose range tested was 0.75 to 12 mg/kg. The NCE/polychromatic erythrocyte (PCE) ratios in peripheral blood were not significantly altered by chemical treatment in the 3-month studies, indicating an absence of induced bone marrow toxicity in this group of animals. However, the NCE/PCE ratios in peripheral blood of Tg.AC mice treated for 6 months were significantly altered in the 6 and 12 mg/kg groups. Male and female mice in these dosed groups had markedly elevated levels of immature PCEs in their blood, implying a stimulation of erythropoiesis, perhaps in response to pentaerythritol triacrylate-induced toxicity.

DISCUSSION AND CONCLUSIONS

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

Monofunctional acrylates, such as ethyl acrylate, and multifunctional acrylates, such as pentaerythritol triacrylate and trimethylolpropane 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 both pentaerythritol triacrylate and trimethylolpropane triacrylate for toxicity and carcinogenicity. Results of the trimethylolpropane 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 pentaerythritol triacrylate are at risk of exposure (Parker and Turk, 1983). However, the widespread use of these compounds in the manufacture of consumer products such as latex paints and floor polishes 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. Pentaerythritol triacrylate was not detected as a sensitizer in repeated-insult patch testing or in guinea pigs using the Buehler method. However, pentaerythritol triacrylate was positive in a guinea pig maximization test (Andrews and Clary 1986). The NTP has tested pentaerythritol triacrylate for its ability to induce hypersensitivity and irritancy in a rodent model (Appendix K). Pentaerythritol triacrylate was found to be an irritant in BALB/c mice at concentrations greater than 0.25%. Pentaerythritol triacrylate was found to be a sensitizing agent in BALB/c mice in the mouse ear swelling test at concentrations greater than 0.1% and in the local lymph node assay at concentrations greater than 0.25%. These studies suggest that pentaerythritol triacrylate has the potential to induce contact hypersensitivity responses in rodents and humans.

Repeated dermal exposure to acrylates including pentaerythritol 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 pentaerythritol 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 dermal chronic inflammation in male and female rats and mice. Rats had slightly more severe skin lesions than mice in the 2-week and 3-month studies. The systemic toxicity was minimal based on the results from clinical pathology, histopathology, and organ weight changes. In rats, a no-observed-adverse-effect level (NOAEL) for dermal lesions was not attained; the NOAEL for dermal effects was determined to be 0.75 mg/kg for male mice and less than 0.75 mg/kg for female mice.

The dose-related skin effects such as irritation, inflammation, and sustained hyperplasia at the site of application in rats and mice in the 3-month studies suggested pentaerythritol 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). The NTP typically performs carcinogenicity studies in two species, rats and mice. Because skin was the only major 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 thought to be an appropriate model for skin squamous cell cancer development in humans because of evidence that the 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).

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 Tg.AC mouse model was showing promise for carcinogenicity testing (hazard identification) via dermal exposure. Efforts were under way to assess more fully the model's potential. The major route of human exposure to pentaerythritol triacrylate is via the skin. 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 and provided an excellent test of the model.

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 neoplasms 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). The Tg.AC mouse model's usefulness and limitations in detecting chemical carcinogens have been evaluated and published (Spalding *et al.*, 2000; Eastin *et al.*, 2001; Tennant *et al.*, 2001; Pritchard *et al.*, 2003, Sistare *et al.*, 2002).

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 they may be 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 pentaerythritol triacrylate at the site of application in the Tg.AC mice. The increased incidences of squamous cell papilloma were dose related in males and females. The incidences of papilloma were significantly increased in 3, 6, and 12 mg/kg males and females. Squamous cell carcinomas occurred in a few animals and the presence at the base of a papilloma in some suggested they arose from the papilloma. Increased incidences of nonneoplastic lesions also occurred at the site of application and included mild chronic active inflammation, hyperplasia of the epidermis, and hyperkeratosis in males and females.

The carcinogenic potential of pentaerythritol triacrylate was evaluated in three previous dermal studies in C3H/HeJ mice. DePass *et al.* (1985) found no tumors in treated animals. The number of grossly visible liver neoplasms was significantly increased in another study in which no treatment-related tumors were found on the skin; only gross lesions were examined histopathologically, and therefore, the actual incidence of liver neoplasms was not determined (Union Carbide, 1988; NCI, 1987). Dermal carcinogenicity studies on eight multifunctional acrylates including pentaerythritol triacrylate were conducted by the Celanese Corporation in C3H/HeJ mice (Andrews and Clary, 1986). Pentaerythritol triacrylate was administered to the intrascapular region of the skin twice weekly at a dose concentration of 100 mg/kg in mineral oil for 80 weeks. Slightly epilated and crusted skin with acanthosis and fibrosis was noted, but no papillomas were induced. Six of 50 mice treated with pentaerythritol triacrylate were reported to have lymphomas; these were not verified on subsequent reexamination (NCI, 1987). No systemic neoplasms related to pentaerythritol triacrylate administration were observed in the current study. The absence of carcinogenic activity in the Celanese studies could be due to the differences in experimental design and strain

of mouse used. The dose concentration, 100 mg/kg, was more than eight times the highest dose (12 mg/kg) in the current study. 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).

Because the Tg.AC mouse model is a reporter phenotype used to predict potential systemic carcinogenicity in humans, it is important to know if the material is likely to be absorbed via dermal exposure in humans. Because of nonavailability of radiolabeled material, it was not determined if, or to what extent, pentaerythritol triacrylate was absorbed via the skin in the current study. There was, however, indirect evidence that pentaerythritol triacrylate would likely be absorbed. Absorption studies of trimethylolpropane triacrylate were successful, and trimethylolpropane triacrylate was absorbed via the dermal route in the Tg.AC mouse (NTP, 2005). Also, systemic lesions including chronic inflammation, hematopoietic cell proliferation, and myelodysplasia may have indicated some systemic exposure; however, these lesions may also have been secondary to the observed skin lesions.

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 the doses that also induced considerable nonneoplastic pathology. Interestingly, ethyl acrylate was negative when dermally administered to the Tg.AC mouse (Nylander-French and French, 1998) and positive when given by gavage to another short-term *ras* model, the *ras*-H2 mouse (Yamamoto *et al.*, 1998). Methyl methacrylate was not carcinogenic in rats or conventional mice when administered by 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, including pentaerythritol triacrylate, suggest that carcinogenic activity of acrylates is expressed at the site of application only.

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 papillomas (skin reporter phenotype) within weeks. In the current 6-month study, pentaerythritol triacrylate induced dose-related increases in the incidence of squamous cell papilloma, with neoplasms appearing as early as 9 weeks. Some neoplasms progressed to carcinoma by the end of the study. Two other multifunctional acrylates, tripropylene glycol diacrylate (Nylander-French and French, 1998) and trimethylolpropane triacrylate (NTP, 2005) induced skin papillomas in this model as well, 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.

Pentaerythritol triacrylate was demonstrated to be a skin irritant and contact sensitizer, while trimethylolpropane triacrylate was characterized as an irritant with much weaker sensitizing potential than pentaerythritol triacrylate (NTP, 2005). Both compounds resulted in a positive papilloma response in the Tg.AC mouse. A review of the literature suggests that other known skin sensitizers are also positive when tested in the Tg.AC model including tripropylene glycol diacrylate, which, like trimethylolpropane triacrylate and pentaerythritol triacrylate, is a multifunctional acrylate (Nylander-French and French, 1998).

Resorcinol was nonmutagenic in *Salmonella* studies and was negative in 2-year NTP gavage studies in F344/N rats and B6C3F$_1$ mice (NTP, 1992). It is known to produce skin sensitization in humans and mice, and has been used to produce skin peeling in humans (Harvey, 1980). In topical studies in the Tg.AC mouse, resorcinol gave a strong skin papilloma response in males and females. Dinitrofluorobenzene is another contact sensitizer that induced neoplasms in the Tg.AC mouse (Albert *et al.*, 1996). In this study, the corticosteroid fluocinolone acetonide was used to block the cell mediated immune response; however, tumor induction still occurred. The authors concluded that cytotoxic property of dinitrofluorobenzene likely accounted for the tumorigenic response in this study.

In addition, based on positivity in the local lymph node assay, there are several other contact sensitizers (NTP, 1999) that were reviewed for tumor response in the Tg.AC mouse by Pritchard *et al.* (2003). A number of these chemicals were positive in the Tg.AC mouse,

including pentachlorophenol, benzo[*a*]pyrene, and 7,12-dimethylbenzanthracene. The relationship is not straightforward though, as pyridine is a sensitizer that was negative in the transgenic mouse assay.

Contact sensitizers are generally electrophilic chemicals that produce protein adduction. Suggestive evidence of an overlap with carcinogens comes from structure-activity alerts for contact sensitizers (Barratt *et al.*, 1994) and carcinogens (Ashby and Tennant, 1994). The possible relationship between induction of skin sensitization and activation of the transgene in Tg.AC mice may merit further study.

An increase in cell replication enhances all steps of neoplastic transformation and tumor development (Enzmann *et al.*, 1998). However, in NTP dermal carcinogenicity studies on rotenone in Tg.AC mice, no papillomas were observed despite the extensive hyperplasia and inflammation in skin at the site of application (Eastin *et al.*, 1998). This result suggests that the mechanism of skin papilloma formation is not yet clearly understood. Repeated dermal administration of pentaerythritol triacrylate in current studies caused sustained epidermal cell proliferation that led to formation of papillomas, a major characteristic of the known neoplasm promoters.

A systemic change was diagnosed in five 12 mg/kg male mice in the current 6-month pentaerythritol triacrylate study that 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 (epididymis, kidney, spleen, heart, lung, lymph nodes, and thymus) 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 mandibular lymph node 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 lack of mutagenic activity with pentaerythritol triacrylate in the *Salmonella* assay (Zeiger *et al.*, 1987) combined with the negative results in the 3-month peripheral blood micronucleus test supports 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), along with the significant increase of micronucleated normochromatic erythrocytes seen in female Tg.AC mice treated with pentaerythritol triacrylate for 6 months (Appendix C), also indicate a potential for a genotoxic mode of action. Therefore, the available evidence does not permit classification of pentaerythritol triacrylate as either a genotoxic or a nongenotoxic carcinogen. Additional studies are needed to understand the mechanism of skin neoplasm formation by pentaerythritol triacrylate.

CONCLUSIONS

Male and female Tg.AC hemizygous mice dosed with pentaerythritol triacrylate for 6 months had significantly increased incidences of squamous cell papilloma of the skin at the site of dermal application. Treatment-related squamous cell carcinomas occurred at the site of application in male mice.

Nonneoplastic lesions noted at the site of application included hyperkeratosis, chronic active inflammation, and epidermal hyperplasia. A hematopoietic disorder (myelodysplasia) occurred in dosed male mice.

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

TABLE A1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol 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			1	
Natural deaths	3				2	5
Survivors						
Terminal sacrifice	12	14	15	15	12	10
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Salivary glands	(1)	(1)		(1)		(2)
Carcinoma	1 (100%)	1 (100%)		1 (100%)		1 (50%)
Stomach, forestomach	(15)	(15)	(15)	(15)	(15)	(15)
Squamous cell papilloma	2 (13%)	3 (20%)	5 (33%)	5 (33%)	5 (33%)	3 (20%)
Squamous cell papilloma, multiple	4 (27%)	1 (7%)		3 (20%)		2 (13%)
Tooth	(2)		(3)	(1)	(3)	(2)
Odontogenic tumor	2 (100%)		3 (100%)	1 (100%)	3 (100%)	2 (100%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Spleen	(15)	(15)	(15)	(15)	(15)	(15)
Integumentary System						
Skin	(15)	(15)	(15)	(15)	(15)	(15)
Squamous cell papilloma, multiple						1 (7%)
Site of application, squamous cell carcinoma				2 (13%)		2 (13%)
Site of application, squamous cell carcinoma, multiple						1 (7%)
Site of application, squamous cell papilloma	1 (7%)		4 (27%)	4 (27%)		
Site of application, squamous cell papilloma, multiple				11 (73%)	15 (100%)	13 (87%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol 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)
Alveolar/bronchiolar adenoma	2 (13%)	1 (7%)				
Carcinoma, metastatic, salivary glands		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%)
Neoplasm Summary						
Total animals with primary neoplasms[c]	9	5	9	15	15	13
Total primary neoplasms	12	6	12	27	23	26
Total animals with benign neoplasms	7	5	8	15	15	13
Total benign neoplasms	9	5	9	23	20	19
Total animals with malignant neoplasms	1	1		3		5
Total malignant neoplasms	1	1		3		5
Total animals with metastatic neoplasms		1				
Total metastatic neoplasms		1				
Total animals with uncertain neoplasms-benign or malignant	2		3	1	3	2
Total uncertain neoplasms	2		3	1	3	2

[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 Pentaerythritol Triacrylate: Vehicle Control

Number of Days on Study	1 3 2	1 4 5	1 5 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 6	1 8 6	1 8 6	1 8 6	
Carcass ID Number	0 0 6	0 1 2	0 1 5	0 0 1	0 0 2	0 0 3	0 0 5	0 0 7	0 1 0	0 1 3	0 1 4	0 0 4	0 0 8	0 0 9	0 1 1	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			X					X			4
Tooth			+		+											2
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																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	M	M	+	+	+	+	M	+	+	+	+	+	+	+	+	12
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Site of application, squamous cell papilloma													X			1
Musculoskeletal System																
None																
Nervous System																
None																

+: 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 Pentaerythritol Triacrylate: Vehicle Control

Number of Days on Study	1 3 2	1 4 5	1 5 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 6	1 8 6	1 8 6	1 8 6	
Carcass ID Number	0 0 6	0 1 2	0 1 5	0 0 1	0 0 2	0 0 3	0 0 5	0 0 7	0 1 0	0 1 3	0 1 4	0 0 4	0 0 8	0 0 9	0 1 1		Total Tissues/ Tumors
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Alveolar/bronchiolar adenoma		X		X													2
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 Pentaerythritol Triacrylate: 0.75 mg/kg

Number of Days on Study	129	185	185	185	185	185	185	185	186	186	186	186	186	186	186	
Carcass ID Number	0025	0018	0019	0020	0023	0024	0026	0030	0016	0117	0021	0022	0027	0028	0029	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
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pituitary gland	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	14
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+		14
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
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
Carcinoma, metastatic, salivary glands	X															1

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

Number of Days on Study	1 2 9	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 6	1 8 6	1 8 6	1 8 6	1 8 6	1 8 6	1 8 6		
Carcass ID Number	0 2 5	0 1 8	0 1 9	0 2 0	0 2 3	0 2 4	0 2 6	0 3 0	0 1 6	0 1 7	0 2 1	0 2 2	0 2 7	0 2 8	0 2 9		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 Pentaerythritol Triacrylate: 1.5 mg/kg

Number of Days on Study	185	185	185	185	185	185	185	185	185	185	186	186	186	186	186	
Carcass ID Number	03131	03232	03333	03434	03838	03939	04040	04443	04444	04045	04355	04356	04367	04411	04412	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma	X	X						X				X		X		5
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	+	+	+	+	+	14
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Site of application, squamous cell papilloma		X	X			X			X							4
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 Pentaerythritol Triacrylate: 1.5 mg/kg

Number of Days on Study	185	185	185	185	185	185	185	185	185	185	186	186	186	186	186	186	
Carcass ID Number	03131	03232	03333	03434	03838	03939	04040	04443	04444	04445	04355	03356	03356	03757	04141	04242	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 Pentaerythritol Triacrylate: 3 mg/kg

Number of Days on Study	185	185	185	185	185	185	185	185	186	186	186	186	186	186	186	186	
Carcass ID Number	04646	04647	04648	05052	05053	05056	05057	05058	04649	05050	05051	05054	05055	05059	05060		Total Tissues/ Tumors
Alimentary System																	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Salivary glands												+					1
Carcinoma												X					1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Squamous cell papilloma			X				X					X	X			X	5
Squamous cell papilloma, multiple									X	X				X			3
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	M	+	+		13
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Integumentary System																	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Site of application, squamous cell carcinoma					X			X									2
Site of application, squamous cell papilloma		X		X										X		X	4
Site of application, squamous cell papilloma, multiple	X		X		X	X	X	X	X	X	X	X			X		11
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 Pentaerythritol Triacrylate: 3 mg/kg

Number of Days on Study	185	185	185	185	185	185	185	185	186	186	186	186	186	186	186	186	
Carcass ID Number	04646	04747	04848	05252	05353	05656	05757	05858	04949	05050	05551	05552	05554	05555	05959	06060	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 Pentaerythritol Triacrylate: 6 mg/kg

Number of Days on Study	163	166	184	185	185	185	185	185	185	185	186	186	186	186	186	
Carcass ID Number	06301	06612	06624	06646	06667	06678	06688	06699	07001	07011	06615	07022	07033	07044	07055	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma						X					X	X	X	X		5
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	14
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
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 A2
Individual Animal Tumor Pathology of Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol Triacrylate: 6 mg/kg

Number of Days on Study	163	166	184	185	185	185	185	185	185	185	185	186	186	186	186	186	
Carcass ID Number	06363	06611	06612	06644	06666	06677	06688	06699	06670	07071	07071	06615	07072	07073	07074	07075	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 Pentaerythritol Triacrylate: 12 mg/kg

Number of Days on Study	1 3 8	1 6 0	1 6 7	1 7 0	1 8 2	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 5	1 8 6	1 8 6	
Carcass ID Number	0 8 8	0 9 0	0 8 9	0 8 7	0 7 9	0 7 6	0 7 7	0 8 0	0 8 1	0 8 3	0 8 4	0 8 5	0 8 6	0 7 8	0 8 2	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	X							2
Tooth				+							+					2
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																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Preputial gland							+									1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Hematopoietic System																
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	14
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	14
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma, multiple									X							1
Site of application, squamous cell carcinoma						X						X				2
Site of application, squamous cell carcinoma, multiple					X											1
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																

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

Number of Days on Study	138	160	167	170	182	185	185	185	185	185	185	185	185	186	186	
Carcass ID Number	08888	08909	08987	08877	07979	07766	07767	08070	08881	08883	08884	08885	08886	07878	08882	Total Tissues/ Tumors
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
None																
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
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 Pentaerythritol Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Lung: Alveolar/bronchiolar Adenoma						
Overall rate[a]	2/15 (13%)	1/15 (7%)	0/15 (0%)	0/15 (0%)	0/15 (0%)	0/15 (0%)
Adjusted rate[b]	14.3%	7.0%	0.0%	0.0%	0.0%	0.0%
Terminal rate[c]	1/12 (8%)	1/14 (7%)	0/15 (0%)	0/15 (0%)	0/12 (0%)	0/10 (0%)
First incidence (days)	145	185 (T)	—[e]	—	—	—
Poly-3 test[d]	P=0.133N	P=0.490N	P=0.215N	P=0.215N	P=0.224N	P=0.238N
Skin: Squamous Cell Papilloma						
Overall rate	1/15 (7%)	0/15 (0%)	4/15 (27%)	15/15 (100%)	15/15 (100%)	13/15 (87%)
Adjusted rate	7.4%	0.0%	26.7%	100.0%	100.0%	86.7%
Terminal rate	1/12 (8%)	0/14 (0%)	4/15 (27%)	15/15 (100%)	12/12 (100%)	8/10 (80%)
First incidence (days)	185 (T)	—	185 (T)	185 (T)	163	138
Poly-3 test	P<0.001	P=0.487N	P=0.198	P<0.001	P<0.001	P<0.001
Skin: Squamous Cell Carcinoma						
Overall rate	0/15 (0%)	0/15 (0%)	0/15 (0%)	2/15 (13%)	0/15 (0%)	3/15 (20%)
Adjusted rate	0.0%	0.0%	0.0%	13.3%	0.0%	22.1%
Terminal rate	0/12 (0%)	0/14 (0%)	0/15 (0%)	2/15 (13%)	0/12 (0%)	2/10 (20%)
First incidence (days)	—	—[f]	—	185 (T)	—	182
Poly-3 test	P=0.011	—	—	P=0.257	—	P=0.106
Skin: Squamous Cell Papilloma or Squamous Cell Carcinoma						
Overall rate	1/15 (7%)	0/15 (0%)	4/15 (27%)	15/15 (100%)	15/15 (100%)	13/15 (87%)
Adjusted rate	7.4%	0.0%	26.7%	100.0%	100.0%	86.7%
Terminal rate	1/12 (8%)	0/14 (0%)	4/15 (27%)	15/15 (100%)	12/12 (100%)	8/10 (80%)
First incidence (days)	185 (T)	—	185 (T)	185 (T)	163	138
Poly-3 test	P<0.001	P=0.487N	P=0.198	P<0.001	P<0.001	P<0.001
Stomach (Forestomach): Squamous Cell Papilloma						
Overall rate	6/15 (40%)	4/15 (27%)	5/15 (33%)	8/15 (53%)	5/15 (33%)	5/15 (33%)
Adjusted rate	43.0%	26.7%	33.3%	53.3%	34.8%	37.0%
Terminal rate	5/12 (42%)	3/14 (21%)	5/15 (33%)	8/15 (53%)	5/12 (42%)	5/10 (50%)
First incidence (days)	145	129	185 (T)	185 (T)	185 (T)	185 (T)
Poly-3 test	P=0.550	P=0.300N	P=0.441N	P=0.429	P=0.474N	P=0.526N
Tooth: Odontogenic Tumor						
Overall rate	2/15 (13%)	0/15 (0%)	3/15 (20%)	1/15 (7%)	3/15 (20%)	2/15 (13%)
Adjusted rate	14.5%	0.0%	20.0%	6.7%	20.8%	14.6%
Terminal rate	1/12 (8%)	0/14 (0%)	3/15 (20%)	1/15 (7%)	2/12 (17%)	1/10 (10%)
First incidence (days)	155	—	185 (T)	185 (T)	184	170
Poly-3 test	P=0.358	P=0.223N	P=0.538	P=0.472N	P=0.519	P=0.697
All Organs: Benign Neoplasms						
Overall rate	7/15 (47%)	5/15 (33%)	8/15 (53%)	15/15 (100%)	15/15 (100%)	13/15 (87%)
Adjusted rate	50.2%	33.3%	53.3%	100.0%	100.0%	86.7%
Terminal rate	6/12 (50%)	4/14 (29%)	8/15 (53%)	15/15 (100%)	12/12 (100%)	8/10 (80%)
First incidence (days)	145	129	185 (T)	185 (T)	163	138
Poly-3 test	P<0.001	P=0.297N	P=0.578	P<0.001	P<0.001	P=0.033

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol 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%)	1/15 (7%)	0/15 (0%)	3/15 (20%)	0/15 (0%)	5/15 (33%)
Adjusted rate	7.4%	6.7%	0.0%	20.0%	0.0%	34.7%
Terminal rate	1/12 (8%)	0/14 (0%)	0/15 (0%)	3/15 (20%)	0/12 (0%)	2/10 (20%)
First incidence (days)	185 (T)	129	—	185 (T)	—	138
Poly-3 test	P=0.008	P=0.736N	P=0.478N	P=0.340	P=0.486N	P=0.094
All Organs: Benign or Malignant Neoplasms						
Overall rate	9/15 (60%)	5/15 (33%)	9/15 (60%)	15/15 (100%)	15/15 (100%)	13/15 (87%)
Adjusted rate	62.7%	33.3%	60.0%	100.0%	100.0%	86.7%
Terminal rate	7/12 (58%)	4/14 (29%)	9/15 (60%)	15/15 (100%)	12/12 (100%)	8/10 (80%)
First incidence (days)	145	129	185 (T)	185 (T)	163	138
Poly-3 test	P<0.001	P=0.108N	P=0.589N	P=0.008	P=0.008	P=0.137

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for lung and 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 Pentaerythritol 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			1	
Natural deaths	3				2	5
Survivors						
Terminal sacrifice	12	14	15	15	12	10
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Atrophy	1 (7%)					
Hematopoietic cell proliferation					2 (13%)	
Infiltration cellular					5 (33%)	3 (20%)
Inflammation, chronic active	5 (33%)	11 (73%)	10 (67%)	13 (87%)	13 (87%)	5 (33%)
Mineralization	1 (7%)					
Myelodysplasia						5 (33%)
Pigmentation		1 (7%)	1 (7%)			
Hepatocyte, necrosis	1 (7%)		1 (7%)		1 (7%)	1 (7%)
Hepatocyte, vacuolization cytoplasmic			1 (7%)			
Salivary glands	(1)	(1)		(1)		(2)
Parotid gland, infiltration cellular						1 (50%)
Stomach, forestomach	(15)	(15)	(15)	(15)	(15)	(15)
Hyperkeratosis	2 (13%)		1 (7%)		3 (20%)	4 (27%)
Epithelium, hyperplasia			1 (7%)			
Cardiovascular System						
Heart	(15)	(15)	(15)	(15)	(15)	(15)
Infiltration cellular						3 (20%)
Inflammation, chronic active		3 (20%)			1 (7%)	
Myelodysplasia						3 (20%)
Endocrine System						
Adrenal cortex	(15)	(15)	(15)	(15)	(15)	(15)
Hypertrophy	7 (47%)	10 (67%)	3 (20%)	5 (33%)	1 (7%)	3 (20%)
Myelodysplasia						2 (13%)
Adrenal medulla	(15)	(15)	(15)	(15)	(15)	(15)
Infiltration cellular						2 (13%)
Myelodysplasia						2 (13%)
Pituitary gland	(15)	(14)	(15)	(15)	(15)	(15)
Pars distalis, cyst	1 (7%)				3 (20%)	
Thyroid gland	(15)	(15)	(15)	(15)	(15)	(15)
Inflammation, chronic active					1 (7%)	
Myelodysplasia						1 (7%)

[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 Pentaerythritol Triacrylate

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
General Body System						
None						
Genital System						
Epididymis	(15)	(14)	(15)	(15)	(15)	(15)
Infiltration cellular					5 (33%)	5 (33%)
Inflammation, chronic active		1 (7%)				4 (27%)
Myelodysplasia						5 (33%)
Bilateral, hypospermia						1 (7%)
Unilateral, hypospermia	1 (7%)	1 (7%)				2 (13%)
Preputial gland						(1)
Duct, ectasia						1 (100%)
Testes	(15)	(15)	(15)	(15)	(15)	(15)
Cyst	2 (13%)	1 (7%)		1 (7%)	1 (7%)	
Bilateral, germinal epithelium, degeneration					1 (7%)	
Unilateral, germinal epithelium, degeneration	1 (7%)	1 (7%)		2 (13%)	1 (7%)	4 (27%)
Hematopoietic System						
Bone marrow						(1)
Myeloid cell, hyperplasia						1 (100%)
Lymph node	(14)	(15)	(14)	(13)	(15)	(14)
Hyperplasia					1 (7%)	
Infiltration cellular, plasma cell					1 (7%)	
Inflammation, chronic active					1 (7%)	
Myelodysplasia						1 (7%)
Axillary, hyperplasia, lymphoid						1 (7%)
Axillary, infiltration cellular					1 (7%)	1 (7%)
Axillary, infiltration cellular, plasma cell					1 (7%)	1 (7%)
Axillary, infiltration cellular, histiocyte						1 (7%)
Inguinal, myelodysplasia						1 (7%)
Mediastinal, hyperplasia					1 (7%)	
Mediastinal, infiltration cellular					4 (27%)	2 (14%)
Mediastinal, myelodysplasia						1 (7%)
Lymph node, mandibular	(15)	(15)	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation					3 (20%)	
Hyperplasia			2 (13%)	1 (7%)	2 (13%)	
Hyperplasia, plasma cell						1 (7%)
Infiltration cellular					5 (33%)	6 (40%)
Infiltration cellular, plasma cell					1 (7%)	6 (40%)
Myelodysplasia						2 (13%)
Necrosis, lymphoid					2 (13%)	
Lymph node, mesenteric	(12)	(15)	(15)	(15)	(15)	(14)
Hematopoietic cell proliferation					1 (7%)	1 (7%)
Infiltration cellular					4 (27%)	2 (14%)
Myelodysplasia						3 (21%)
Necrosis, lymphoid					1 (7%)	
Spleen	(15)	(15)	(15)	(15)	(15)	(15)
Atrophy	1 (7%)					
Depletion cellular					2 (13%)	2 (13%)
Hematopoietic cell proliferation	1 (7%)	2 (13%)	1 (7%)	2 (13%)	9 (60%)	8 (53%)
Infiltration cellular					1 (7%)	4 (27%)
Myelodysplasia						4 (27%)
Necrosis, lymphoid					2 (13%)	1 (7%)

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

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Hematopoietic System (continued)						
Thymus	(15)	(15)	(15)	(15)	(14)	(14)
Atrophy	4 (27%)	1 (7%)	2 (13%)		4 (29%)	5 (36%)
Myelodysplasia						3 (21%)
Thymocyte, necrosis					2 (14%)	
Integumentary System						
Skin	(15)	(15)	(15)	(15)	(15)	(15)
Dermis, site of application, necrosis						1 (7%)
Epidermis, site of application, hyperplasia			3 (20%)	8 (53%)	10 (67%)	14 (93%)
Epidermis, site of application, ulcer					1 (7%)	
Site of application, cyst epithelial inclusion						1 (7%)
Site of application, hyperkeratosis	1 (7%)		2 (13%)	6 (40%)	10 (67%)	13 (87%)
Site of application, inflammation, chronic active				2 (13%)	5 (33%)	12 (80%)
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(15)
Infiltration cellular					1 (7%)	3 (20%)
Inflammation, chronic active					1 (7%)	2 (13%)
Myelodysplasia						3 (20%)
Alveolar epithelium, hyperplasia			1 (7%)			
Mediastinum, infiltration cellular						1 (7%)
Special Senses System						
None						
Urinary System						
Kidney	(15)	(15)	(15)	(15)	(15)	(15)
Hydronephrosis						1 (7%)
Infarct			1 (7%)	2 (13%)		
Infiltration cellular					2 (13%)	3 (20%)
Inflammation, chronic active	1 (7%)		4 (27%)	4 (27%)	3 (20%)	
Mineralization					1 (7%)	
Myelodysplasia						4 (27%)
Nephropathy	6 (40%)	1 (7%)				
Pigmentation	1 (7%)					
Cortex, cyst		1 (7%)		1 (7%)	1 (7%)	1 (7%)
Renal tubule, dilatation				1 (7%)		
Renal tubule, regeneration	1 (7%)	2 (13%)		3 (20%)		

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 Pentaerythritol Triacrylate: 3 mg/kg[a]

Carcass ID Number	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	Total Tumors	Animals with Tumors
Week																	
14	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1
15	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	2	2
16	0	0	0	0	0	0	0	2	0	0	0	2	3	1	0	8	4
17	0	0	0	0	0	0	0	1	0	0	0	2	2	1	0	6	4
18	0	0	0	0	0	0	0	1	0	0	4	2	2	1	0	10	5
19	0	0	0	0	0	0	0	0	0	0	3	2	2	1	1	9	5
20	0	0	1	4	0	0	0	0	0	0	3	2	2	1	1	14	7
21	0	0	2	5	2	0	0	0	3	0	2	4	1	1	1	21	9
22	0	0	2	6	9	3	0	0	3	0	3	4	1	1	1	33	10
23	0	0	2	6	9	3	0	0	2	0	4	5	1	1	1	34	10
24	0	0	1	7	11	4	0	0	5	0	5	8	1	1	1	44	10
25	2	0	2	6	11	4	0	0	5	0	9	7	1	2	1	50	11
26	2	0	3	8	12	3	1	0	5	0	10	9	1	3	1	58	12
27	2	0	3	7	10	6	1	0	5	0	11	11	2	3	1	62	12
Necropsy	2	1	3	8	14	8	1	6	7	2	10	13	3	3	1	89	15

X=Animal died

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 Pentaerythritol Triacrylate: 6 mg/kg

Carcass ID Number	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	Total Tumors	Animals with Tumors
Week																	
9	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
10	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	3	2
11	2	0	2	0	0	0	0	0	0	0	1	0	0	0	0	5	3
12	2	0	2	2	4	0	0	0	0	2	1	0	0	0	0	13	6
13	6	3	3	2	5	0	0	2	0	4	1	2	1	0	0	29	10
14	6	3	5	6	3	2	0	2	3	7	1	3	4	4	0	49	13
15	7	3	6	7	3	3	5	2	2	9	2	5	4	7	5	70	15
16	9	3	7	7	4	4	6	3	3	7	4	8	5	4	8	82	15
17	17	3	13	9	6	8	9	6	2	12	3	6	5	10	10	119	15
18	20	5	15	11	13	10	9	6	5	15	5	16	6	11	20	≥167	15
19	20	5	16	13	14	15	11	7	5	20	6	20	5	13	18	≥188	15
20	20	6	20	17	14	17	12	11	4	20	12	20	9	11	20	≥213	15
21	20	6	20	15	18	20	16	11	4	20	12	20	14	15	20	≥231	15
22	20	8	20	20	17	20	20	12	10	20	15	20	15	20	20	≥257	15
23	20	15	20	20	20	20	20	15	13	20	14	20	14	20	20	≥271	15
24	20	20	20	20	20	20	20	20	16	20	20	20	20	20	20	≥296	15
25	X	20	X	20	20	20	20	17	17	20	20	20	15	20	20	≥249	13
26		20		20	20	20	20	20	20	20	20	20	20	20	20	≥260	13
27		20		20	20	20	20	20	20	20	20	20	20	20	20	≥260	13
Necropsy	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	≥300	15

X=Animal died

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 Pentaerythritol Triacrylate: 12 mg/kg

Carcass ID Number	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	Total Tumors	Animals with Tumors
Week																	
9	4	0	0	0	0	3	0	0	4	4	1	2	0	0	0	18	6
10	4	0	0	2	3	5	0	0	8	13	1	2	0	8	0	46	9
11	4	0	0	4	6	4	0	3	9	15	1	2	3	10	0	61	11
12	6	0	0	9	10	10	0	4	14	20	4	2	7	15	13	≥114	12
13	7	7	0	10	11	14	0	6	14	20	5	2	6	20	20	≥142	13
14	7	10	0	11	12	9	0	6	14	20	4	2	7	20	20	≥142	13
15	11	12	0	20	20	10	0	6	15	20	4	3	13	20	20	≥174	13
16	10	13	0	20	16	15	0	6	16	20	6	3	16	20	20	≥181	13
17	18	15	0	18	20	20	0	7	20	20	10	4	20	20	20	≥212	13
18	20	20	0	20	20	20	0	12	20	20	17	4	20	20	20	≥233	13
19	20	20	0	20	20	20	0	15	20	20	12	7	20	20	20	≥234	13
20	20	20	0	20	20	20	0	15	20	20	15	12	20	20	20	≥242	13
21	20	20	0	20	20	20	0	20	20	20	20	14	X	20	20	≥234	12
22	20	20	0	20	20	20	0	20	20	20	20	20		20	20	≥240	12
23	20	20	0	20	20	20	0	20	20	20	20	20		20	20	≥240	12
24	20	20	0	20	20	20	0	20	20	20	20	20		20	X	≥220	11
25	20	20	0	20	20	20	0	20	20	20	20	20		X		≥200	10
26	20	20	0	20	20	20	0	20	20	20	20	X				≥180	9
27	20	20	0	X	20	20	0	20	20	20	20					≥160	8
Necropsy	20	20	0	20	20	20	0	20	20	20	20	20	20	20	20	≥260	13

X=Animal died

[a] Animal 8 in the vehicle control group had a single papilloma first observed at necropsy. No papillomas occurred in the 0.75 mg/kg group. In the 1.5 mg/kg group, animal 32 had a single papilloma observed only at week 21, and animal 39 had a single papilloma observed from week 20 through necropsy. 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 62 in the 6 mg/kg group was sacrificed one day before study termination.

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

TABLE B1
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol 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	1				3
Natural deaths	2		3	3	2	3
Survivors						
Terminal sacrifice	12	14	12	12	13	9
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Salivary glands						(1)
Carcinoma						1 (100%)
Stomach, forestomach	(15)	(15)	(15)	(15)	(15)	(15)
Squamous cell papilloma	2 (13%)	4 (27%)	5 (33%)	4 (27%)	6 (40%)	3 (20%)
Squamous cell papilloma, multiple	3 (20%)			3 (20%)	1 (7%)	1 (7%)
Tooth	(4)		(3)	(3)	(1)	
Odontogenic tumor	4 (100%)		3 (100%)	2 (67%)	1 (100%)	
Cardiovascular System						
Heart	(15)	(15)	(15)	(15)	(15)	(15)
Endocrine System						
Adrenal cortex	(15)	(15)	(15)	(15)	(15)	(15)
Adrenal medulla	(15)	(15)	(15)	(14)	(15)	(15)
Pheochromocytoma benign			1 (7%)			
Pituitary gland	(15)	(15)	(14)	(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	(15)	(14)	(13)	(12)	(15)	(14)
Lymph node, mandibular	(15)	(15)	(15)	(15)	(15)	(13)
Lymph node, mesenteric	(14)	(15)	(15)	(14)	(15)	(15)
Spleen	(15)	(15)	(15)	(15)	(15)	(15)
Thymus	(15)	(15)	(15)	(14)	(14)	(15)

TABLE B1
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol 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, multiple					1 (7%)	
Site of application, squamous cell carcinoma						1 (7%)
Site of application, squamous cell papilloma				3 (20%)		
Site of application, squamous cell papilloma, multiple			1 (7%)	7 (47%)	12 (80%)	13 (87%)
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(15)
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%)
Lymphoma malignant				1 (7%)		
Neoplasm Summary						
Total animals with primary neoplasms[c]	9	4	6	13	13	14
Total primary neoplasms	10	4	10	20	22	20
Total animals with benign neoplasms	5	4	6	11	13	13
Total benign neoplasms	5	4	7	17	21	17
Total animals with malignant neoplasms	1			1		3
Total malignant neoplasms	1			1		3
Total animals with uncertain neoplasms-benign or malignant	4		3	2	1	
Total uncertain neoplasms	4		3	2	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 Pentaerythritol Triacrylate: Vehicle Control

Number of Days on Study	0 6 3	0 7 0	1 7 7	1 8 6	1 8 6	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	
Carcass ID Number	1 0 0 1	1 0 0 4	1 0 0 0	0 0 9 7	0 0 9 8	0 0 9 1	0 0 9 2	0 0 9 3	0 0 9 4	0 0 9 5	0 0 9 6	0 0 9 9	1 0 2	1 0 3	1 0 5	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma													X		X	2
Squamous cell papilloma, multiple					X							X		X		3
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
Hematopoietic System																
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Lymph node, mesenteric	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	14
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 B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol Triacrylate: Vehicle Control

Number of Days on Study	0 6 3	0 7 0	1 7 7	1 8 6	1 8 6	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	
Carcass ID Number	1 0 1	1 0 4	1 0 0	0 9 7	0 9 8	0 9 1	0 9 2	0 9 3	0 9 4	0 9 5	0 9 6	0 9 9	1 0 2	1 0 3	1 0 5	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 Pentaerythritol Triacrylate: 0.75 mg/kg

Number of Days on Study	0 6 7	1 8 6	1 8 6	1 8 6	1 8 6	1 8 6	1 8 6	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	
Carcass ID Number	1 1 4	1 0 8	1 1 1	1 1 2	1 1 6	1 1 8	1 2 0	1 0 6	1 0 7	1 0 9	1 1 0	1 1 3	1 1 5	1 1 7	1 1 9	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma			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
Hematopoietic System																
Lymph node	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	14
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
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 Pentaerythritol Triacrylate: 1.5 mg/kg

Number of Days on Study	0 0 5	1 0 9	1 4 9	1 8 6	1 8 6	1 8 6	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	
Carcass ID Number	1 3 4	1 2 4	1 2 1	1 2 7	1 3 1	1 3 5	1 2 2	1 2 3	1 2 5	1 2 6	1 2 8	1 2 9	1 3 0	1 3 2	1 3 3	Total Tissues/ Tumors
Alimentary System																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Squamous cell papilloma									X	X	X	X	X			5
Tooth									+	+	+					3
Odontogenic tumor									X	X	X					3
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Pheochromocytoma benign															X	1
Pituitary gland	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	14
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
Site of application, squamous cell papilloma, multiple										X						1
Musculoskeletal System																
None																
Nervous System																
None																
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
None																

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

Number of Days on Study	0 0 5	1 0 9	1 4 9	1 8 6	1 8 6	1 8 6	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	
Carcass ID Number	1 3 4	1 2 4	1 2 1	1 2 7	1 3 1	1 3 5	1 2 2	1 2 3	1 2 5	1 2 6	1 2 8	1 2 9	1 3 0	1 3 2	1 3 3	Total Tissues/ Tumors	
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 Pentaerythritol Triacrylate: 3 mg/kg

Number of Days on Study	167	177	180	186	186	186	186	187	187	187	187	187	187	187	187	
Carcass ID Number	149	136	142	139	140	143	145	137	138	141	144	146	147	148	150	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														2
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Endocrine System																
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Adrenal medulla	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	14
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	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	14
Integumentary System																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Site of application, squamous cell papilloma		X			X									X		3
Site of application, squamous cell papilloma, multiple						X	X	X	X	X	X	X				7
Musculoskeletal System																
Bone	+															1
Nervous System																
None																
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Special Senses System																
None																

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

Number of Days on Study	167	177	180	186	186	186	186	187	187	187	187	187	187	187	187	187	
Carcass ID Number	14 49	13 36	14 02	13 09	14 04	14 43	14 45	13 37	13 38	14 41	14 44	14 46	14 47	14 48	15 00		Total Tissues/ Tumors
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymphoma malignant			X														1

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

Number of Days on Study	0858	1182	1486	1486	1606	1866	1865	1872	1873	1876	1877	1877	1877	1877	1877	1887	
Carcass ID Number	15051	15057	15054	15055	16060	16063	16065	15052	15053	15056	15058	15059	16061	16062	16064		Total Tissues/ Tumors
Alimentary System																	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Squamous cell papilloma	X			X	X		X			X					X		6
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
Vagina					+												1
Squamous cell papilloma					X												1
Hematopoietic System																	
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+		14
Integumentary System																	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		15
Squamous cell papilloma, multiple					X												1
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 B2
Individual Animal Tumor Pathology of Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol Triacrylate: 6 mg/kg

Number of Days on Study	0858	1882	1886	1866	1866	1866	1867	1877	1877	1877	1877	1877	1877	1887	
Carcass ID Number	15151	15157	15154	15155	16060	16063	16065	15652	15653	15656	15858	15859	16061	16062	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 Pentaerythritol Triacrylate: 12 mg/kg

Number of Days on Study	0 2 7	0 9 8	1 1 3	1 2 6	1 3 4	1 6 2	1 8 6	1 8 6	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	1 8 7	
Carcass ID Number	1 7 1	1 6 9	1 7 0	1 7 6	1 6 6	1 7 3	1 6 7	1 7 2	1 6 8	1 7 4	1 7 5	1 7 7	1 7 8	1 7 9	1 8 0	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
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	+	+	+	+	+	+	+	+	+	+	+	+	+	14
Lymph node, mandibular	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	13
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Integumentary System																
Mammary gland				+												1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15
Site of application, squamous cell carcinoma															X	1
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15

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

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

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol 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%)	1/15 (7%)	10/15 (67%)	12/15 (80%)	13/15 (87%)
Adjusted rate[b]	0.0%	0.0%	7.9%	68.4%	85.1%	92.8%
Terminal rate[c]	0/12 (0%)	0/14 (0%)	1/12 (8%)	9/12 (75%)	11/13 (85%)	8/9 (89%)
First incidence (days)	—[e]	—[f]	186 (T)	177	182	98
Poly-3 test[d]	P<0.001	—	P=0.496	P<0.001	P<0.001	P<0.001
Skin: Squamous Cell Carcinoma						
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%	9.3%
Terminal rate	0/12 (0%)	0/14 (0%)	0/12 (0%)	0/12 (0%)	0/13 (0%)	1/9 (11%)
First incidence (days)	—	—	—	—	—	186 (T)
Poly-3 test	P=0.090	—	—	—	—	P=0.462
Skin: Squamous Cell Papilloma or Squamous Cell Carcinoma						
Overall rate	0/15 (0%)	0/15 (0%)	1/15 (7%)	10/15 (67%)	12/15 (80%)	13/15 (87%)
Adjusted rate	0.0%	0.0%	7.9%	68.4%	85.1%	92.8%
Terminal rate	0/12 (0%)	0/14 (0%)	1/12 (8%)	9/12 (75%)	11/13 (85%)	8/9 (89%)
First incidence (days)	—	—	186 (T)	177	182	98
Poly-3 test	P<0.001	—	P=0.496	P<0.001	P<0.001	P<0.001
Stomach (Forestomach): Squamous Cell Papilloma						
Overall rate	5/15 (33%)	4/15 (27%)	5/15 (33%)	7/15 (47%)	7/15 (47%)	4/15 (27%)
Adjusted rate	38.6%	28.5%	39.3%	48.3%	46.9%	37.3%
Terminal rate	5/12 (42%)	4/14 (29%)	5/12 (42%)	7/12 (58%)	6/13 (46%)	4/9 (44%)
First incidence (days)	186 (T)	186 (T)	186 (T)	186 (T)	85	186 (T)
Poly-3 test	P=0.409	P=0.442N	P=0.640	P=0.451	P=0.478	P=0.637N
Tooth: Odontogenic Tumor						
Overall rate	4/15 (27%)	0/15 (0%)	3/15 (20%)	2/15 (13%)	1/15 (7%)	0/15 (0%)
Adjusted rate	30.6%	0.0%	23.6%	13.4%	7.1%	0.0%
Terminal rate	3/12 (25%)	0/14 (0%)	3/12 (25%)	0/12 (0%)	1/13 (8%)	0/9 (0%)
First incidence (days)	177	—	186 (T)	167	186 (T)	—
Poly-3 test	P=0.077N	P=0.036N	P=0.518N	P=0.264N	P=0.139N	P=0.070N
All Organs: Benign Neoplasms						
Overall rate	5/15 (33%)	4/15 (27%)	6/15 (40%)	11/15 (73%)	13/15 (87%)	13/15 (87%)
Adjusted rate	38.6%	28.5%	47.2%	75.2%	86.7%	92.8%
Terminal rate	5/12 (42%)	4/14 (29%)	6/12 (50%)	10/12 (83%)	11/13 (85%)	8/9 (89%)
First incidence (days)	186 (T)	186 (T)	186 (T)	177	85	98
Poly-3 test	P<0.001	P=0.442N	P=0.484	P=0.052	P=0.006	P<0.001
All Organs: Malignant Neoplasms						
Overall rate	1/15 (7%)	0/15 (0%)	0/15 (0%)	1/15 (7%)	0/15 (0%)	3/15 (20%)
Adjusted rate	7.2%	0.0%	0.0%	6.9%	0.0%	24.2%
Terminal rate	0/12 (0%)	0/14 (0%)	0/12 (0%)	0/12 (0%)	0/13 (0%)	1/9 (11%)
First incidence (days)	70	—	—	180	—	27
Poly-3 test	P=0.028	P=0.498N	P=0.518N	P=0.750N	P=0.498N	P=0.254

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol 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	9/15 (60%)	4/15 (27%)	6/15 (40%)	13/15 (87%)	13/15 (87%)	14/15 (93%)
Adjusted rate	64.1%	28.5%	47.2%	86.7%	86.7%	93.3%
Terminal rate	7/12 (58%)	4/14 (29%)	6/12 (50%)	10/12 (83%)	11/13 (85%)	8/9 (89%)
First incidence (days)	70	186 (T)	186 (T)	167	85	27
Poly-3 test	P<0.001	P=0.059N	P=0.314N	P=0.162	P=0.162	P=0.063

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for skin; for all 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 Pentaerythritol 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	1				3
Natural deaths	2		3	3	2	3
Survivors						
Terminal sacrifice	12	14	12	12	13	9
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation				1 (7%)	1 (7%)	6 (40%)
Infiltration cellular					3 (20%)	2 (13%)
Inflammation, chronic active	12 (80%)	14 (93%)	11 (73%)	14 (93%)	13 (87%)	11 (73%)
Necrosis						1 (7%)
Pigmentation	11 (73%)	11 (73%)	9 (60%)	13 (87%)	5 (33%)	2 (13%)
Bile duct, hyperplasia					1 (7%)	
Hepatocyte, necrosis	2 (13%)	2 (13%)	2 (13%)	3 (20%)	4 (27%)	2 (13%)
Stomach, forestomach	(15)	(15)	(15)	(15)	(15)	(15)
Hyperkeratosis	1 (7%)		3 (20%)	3 (20%)	4 (27%)	4 (27%)
Inflammation					2 (13%)	
Epithelium, hyperplasia	1 (7%)				2 (13%)	
Epithelium, ulcer					1 (7%)	
Tooth	(4)		(3)	(3)	(1)	
Inflammation, chronic active				1 (33%)		
Cardiovascular System						
Heart	(15)	(15)	(15)	(15)	(15)	(15)
Infiltration cellular						1 (7%)
Inflammation, chronic active		1 (7%)				1 (7%)
Endocrine System						
Adrenal cortex	(15)	(15)	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation						1 (7%)
Necrosis						1 (7%)
Capsule, hyperplasia	1 (7%)	4 (27%)	7 (47%)	2 (13%)	1 (7%)	2 (13%)
Pituitary gland	(15)	(15)	(14)	(15)	(15)	(15)
Pars distalis, cyst	3 (20%)		2 (14%)	2 (13%)	4 (27%)	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 Pentaerythritol 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%)	2 (13%)	
Uterus	(15)	(15)	(15)	(15)	(15)	(15)
Hydrometra	1 (7%)					2 (13%)
Endometrium, hyperplasia, cystic	11 (73%)	9 (60%)	7 (47%)	10 (67%)	11 (73%)	7 (47%)
Hematopoietic System						
Lymph node	(15)	(14)	(13)	(12)	(15)	(14)
Hematopoietic cell proliferation						2 (14%)
Inguinal, hematopoietic cell proliferation						1 (7%)
Inguinal, hyperplasia						1 (7%)
Inguinal, infiltration cellular						1 (7%)
Inguinal, infiltration cellular, plasma cell						1 (7%)
Mediastinal, hematopoietic cell proliferation						1 (7%)
Mediastinal, hyperplasia						1 (7%)
Mediastinal, infiltration cellular					3 (20%)	1 (7%)
Mediastinal, infiltration cellular, plasma cell						1 (7%)
Mediastinal, necrosis, lymphoid			1 (8%)			
Lymph node, mandibular	(15)	(15)	(15)	(15)	(15)	(13)
Hematopoietic cell proliferation					1 (7%)	8 (62%)
Hyperplasia	3 (20%)		1 (7%)	5 (33%)	3 (20%)	1 (8%)
Infiltration cellular				1 (7%)	2 (13%)	2 (15%)
Infiltration cellular, plasma cell					1 (7%)	1 (8%)
Necrosis, lymphoid		1 (7%)	1 (7%)			
Lymph node, mesenteric	(14)	(15)	(15)	(14)	(15)	(15)
Infiltration cellular					4 (27%)	
Necrosis, lymphoid		1 (7%)				
Spleen	(15)	(15)	(15)	(15)	(15)	(15)
Depletion cellular	2 (13%)	1 (7%)	2 (13%)	1 (7%)	2 (13%)	3 (20%)
Hematopoietic cell proliferation				2 (13%)	11 (73%)	11 (73%)
Infiltration cellular					1 (7%)	1 (7%)
Necrosis, lymphoid		1 (7%)	1 (7%)		1 (7%)	
Thymus	(15)	(15)	(15)	(14)	(14)	(15)
Atrophy	3 (20%)		2 (13%)	2 (14%)	3 (21%)	6 (40%)
Pigmentation				1 (7%)		
Thymocyte, necrosis		1 (7%)	2 (13%)	2 (14%)		5 (33%)
Integumentary System						
Skin	(15)	(15)	(15)	(15)	(15)	(15)
Epidermis, site of application, hyperplasia			5 (33%)	14 (93%)	14 (93%)	14 (93%)
Epidermis, site of application, ulcer						1 (7%)
Sebaceous gland, site of application, hyperplasia, adenomatous					1 (7%)	
Site of application, cyst epithelial inclusion					1 (7%)	1 (7%)
Site of application, hyperkeratosis	1 (7%)		4 (27%)	14 (93%)	11 (73%)	13 (87%)
Site of application, inflammation, chronic active			1 (7%)	9 (60%)	10 (67%)	15 (100%)
Subcutaneous tissue, hemorrhage				1 (7%)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol 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)
Infiltration cellular						1 (7%)
Infiltration cellular, mononuclear cell			1 (7%)			
Inflammation, chronic active				1 (7%)		1 (7%)
Alveolar epithelium, hyperplasia					1 (7%)	
Special Senses System						
None						
Urinary System						
Kidney	(15)	(15)	(15)	(15)	(15)	(15)
Inflammation, chronic active	1 (7%)	2 (13%)	1 (7%)	1 (7%)	1 (7%)	1 (7%)
Nephropathy	3 (20%)					2 (13%)
Polyarteritis	1 (7%)					
Cortex, cyst						2 (13%)
Papilla, necrosis			1 (7%)			
Renal tubule, degeneration						1 (7%)
Renal tubule, hyperplasia			1 (7%)	1 (7%)		
Renal tubule, necrosis						2 (13%)
Renal tubule, regeneration	1 (7%)	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 Pentaerythritol Triacrylate: 3 mg/kg[a]

Carcass ID Number	13 36	13 37	13 38	13 39	14 00	14 01	14 02	14 03	14 04	14 05	14 06	14 07	14 08	14 09	15 00	Total Tumors	Animals with Tumors
Week																	
17	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1
18	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	2	2
19	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	2	2
20	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	3	1
21	0	1	0	0	0	2	0	3	0	0	0	0	0	0	0	6	3
22	0	1	0	0	0	2	0	5	0	2	0	0	1	0	0	11	5
23	0	1	0	0	0	4	0	5	0	3	0	0	1	0	0	14	5
24	0	1	0	0	1	5	0	5	2	4	0	0	1	0	0	19	7
25	0	3	0	0	0	7	0	4	2	4	0	0	1	X	0	21	6
26	1	3	0	0	1	7	0	6	5	4	0	0	1		0	28	8
27	X	3	0	0	1	8	X	4	5	4	0	0	1		0	26	7
Necropsy	1	2	2	0	1	7	0	4	5	4	5	2	1	0	0	34	11

X=Animal died

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 Pentaerythritol Triacrylate: 6 mg/kg

Carcass ID Number	15151	15152	15153	15154	15155	15156	15157	15158	15159	15160	15161	15162	15163	15164	15165	Total Tumors	Animals with Tumors
Week																	
11	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3	4	2
12	1	0	0	0	1	0	0	0	1	2	0	0	8	0	5	18	6
13	0	0	0	4	1	0	0	0	3	2	0	2	10	0	5	27	7
14	X	0	0	4	5	0	0	0	4	2	7	2	10	1	7	42	9
15		0	0	8	7	0	6	0	4	8	9	4	10	2	9	67	10
16		0	0	8	7	0	5	0	6	7	12	4	14	6	8	77	10
17		0	0	7	10	0	10	12	20	15	15	7	20	6	9	≥131	11
18		0	0	10	12	0	20	20	20	13	17	8	20	20	11	≥171	11
19		0	0	13	13	8	18	20	20	10	20	17	20	17	13	≥189	12
20		0	0	14	14	9	20	20	20	20	20	20	20	20	13	≥210	12
21		0	0	15	20	10	20	20	20	20	20	20	20	20	15	≥220	12
22		0	0	20	20	11	20	20	20	20	20	20	20	20	13	≥224	12
23		0	0	20	20	12	20	20	20	20	20	20	20	20	20	≥232	12
24		0	0	20	20	14	20	20	20	20	20	20	20	20	20	≥234	12
25		0	0	20	20	14	20	20	20	20	20	20	20	20	20	≥234	12
26		0	0	20	20	17	20	20	20	20	20	20	20	20	20	≥237	12
27		0	0	20	20	16	X	20	20	20	20	20	20	20	20	≥216	12
Necropsy	0	0	0	20	20	20	20	20	20	20	20	20	20	20	20	≥240	12

X=Animal died

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 Pentaerythritol Triacrylate: 12 mg/kg

Carcass ID Number	16 66	16 67	16 68	16 69	17 00	17 01	17 02	17 03	17 04	17 05	17 06	17 07	17 08	17 09	18 00	Total Tumors	Animals with Tumors
Week																	
5	0	0	0	0	0	X	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0		0	0	0	0	4	0	0	1	0	5	2
10	5	0	0	0	0		0	3	2	0	5	0	5	3	0	23	6
11	4	0	0	0	0		3	4	3	0	5	2	6	4	5	36	9
12	5	9	6	13	10		10	6	4	0	6	5	8	4	11	97	13
13	6	12	5	15	11		10	8	5	0	4	8	9	4	10	107	13
14	10	12	10	15	15		13	8	6	0	10	8	9	5	13	134	13
15	13	15	17	X	15		18	13	11	0	11	15	11	5	20	≥164	12
16	13	20	16		20		20	15	10	0	9	17	13	5	20	≥178	12
17	17	20	20		X		20	20	15	0	9	20	13	5	20	≥179	11
18	20	20	20				20	20	20	0	14	20	20	6	20	≥200	11
19	20	20	20				20	20	20	0	X	20	20	6	20	≥186	10
20	20	20	20				20	20	20	0		20	20	10	20	≥190	10
21	X	20	20				20	20	20	0		20	20	9	20	≥169	9
22		20	20				20	20	20	0		20	20	20	20	≥180	9
23		20	20				20	20	20	0		20	20	15	20	≥175	9
24		20	20				20	X	20	0		20	20	20	20	≥160	8
25		20	20				20		20	0		20	20	17	20	≥157	8
26		20	20				20		20	0		20	20	20	20	≥160	8
27		20	20				20		20	0		20	20	20	20	≥160	8
Necropsy	20	20	20	18	20	0	20	20	20	0	12	20	20	20	20	≥250	13

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 126 had two papillomas first observed at necropsy. 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.

APPENDIX C
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1987). Pentaerythritol triacrylate was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of pentaerythritol triacrylate. In the absence of toxicity, 10,000 µg/plate was selected as the high dose. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

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-month and 6-month studies, peripheral blood samples were obtained from male and female B6C3F$_1$ (3-month) or Tg.AC hemizygous (6-month) mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) per animal. In addition, the PCE/NCE ratio 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 dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). 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 protocols. 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

Pentaerythritol triacrylate (33-10,000 µg/plate) was not mutagenic in *S. typhimurium* strain TA98, TA100, TA1535, or TA1537 with or without Aroclor-induced rat or hamster S9 enzymes (Zeiger *et al.*, 1987; Table C1). No increase in the frequency of micronucleated NCEs was observed in peripheral blood samples from male or female B6C3F_1 mice treated dermally with pentaerythritol triacrylate for 3 months (Table C2). In contrast, treatment of Tg.AC hemizygous female mice administered pentaerythritol triacrylate dermally for 6 months induced a significant increase in micronucleated NCEs; the trend test analysis was highly significant ($P \leq 0.001$), and the two highest dose groups showed mean values significantly elevated over the vehicle control frequency (Table C3). In Tg.AC hemizygous male mice treated with pentaerythritol triacrylate for 6 months, a small increase in micronucleated NCEs was detected; however, the response was judged to be equivocal, due to a positive trend test ($P=0.001$) without any one dose group being significantly elevated over the vehicle control frequency. In both micronucleus studies, the dose range tested was 0.75 to 12 mg pentaerythritol triacrylate/kg body weight. The NCE/PCE ratios in peripheral blood were not significantly altered by chemical treatment in the 3-month study, indicating an absence of induced bone marrow toxicity in this group of animals. However, the NCE/PCE ratios in peripheral blood of Tg.AC mice treated for 6-months were significantly altered at 6 and 12 mg/kg. Male and female mice in these dosed groups had markedly elevated levels of immature PCEs in their blood, implying a stimulation of erythropoiesis, which was perhaps in response to pentaerythritol triacrylate-induced toxicity.

TABLE C1
Mutagenicity of Pentaerythritol Triacrylate in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100							
	0	156 ± 8.7	76 ± 5.0	235 ± 12.5	174 ± 10.7	198 ± 20.5	150 ± 5.0
	100	170 ± 15.3	70 ± 4.7	218 ± 7.2	131 ± 2.3	203 ± 12.2	141 ± 10.7
	333	121 ± 23.8	67 ± 3.0	232 ± 7.4	124 ± 9.6	196 ± 3.1	140 ± 9.8
	1,000	160 ± 5.8	58 ± 3.0	222 ± 10.7	118 ± 2.2	188 ± 5.0	123 ± 5.6
	3,333	142 ± 22.9	57 ± 1.2	223 ± 1.3	110 ± 10.8	191 ± 1.7	106 ± 10.5
	10,000	153 ± 7.9	53 ± 5.5	194 ± 14.6	93 ± 5.6	203 ± 7.8	97 ± 8.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		479 ± 14.1	791 ± 38.1	835 ± 146.6	1,014 ± 63.5	1,858 ± 131.6	2,719 ± 75.4

		–S9 Trial 1	–S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3
TA1535						
	0	22 ± 2.9	7 ± 0.6	24 ± 3.0	10 ± 0.9	9 ± 0.6
	33					7 ± 0.3
	100	20 ± 1.5	6 ± 1.2	42 ± 3.2	12 ± 2.5	11 ± 0.3
	333	24 ± 3.0	9 ± 0.7	39 ± 3.4	13 ± 2.0	13 ± 0.3
	1,000	24 ± 0.6	7 ± 0.9	40 ± 3.1	9 ± 1.2	8 ± 2.4
	3,333	22 ± 1.2	7 ± 0.9	46 ± 2.6	9 ± 2.2	9 ± 0.0
	10,000	18 ± 1.3	6 ± 0.3	33 ± 5.8	10 ± 1.8	7 ± 1.5
Trial summary		Negative	Negative	Equivocal	Negative	Negative
Positive control		1,127 ± 2.1	821 ± 34.0	529 ± 56.4	772 ± 15.1	799 ± 16.3

TA1535 (continued)

		+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
	0	25 ± 4.4	10 ± 1.7	9 ± 1.2
	33			12 ± 1.7
	100	31 ± 2.6	11 ± 1.2	10 ± 0.9
	333	19 ± 3.6	12 ± 2.0	12 ± 0.6
	1,000	35 ± 1.5	14 ± 2.9	9 ± 1.5
	3,333	39 ± 4.0	7 ± 1.5	7 ± 1.0
	10,000	24 ± 6.3	6 ± 0.3	6 ± 1.5
Trial summary		Negative	Negative	Negative
Positive control		997 ± 51.3	704 ± 42.9	834 ± 41.4

TABLE C1
Mutagenicity of Pentaerythritol Triacrylate in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA1537							
	0	6 ± 1.5	7 ± 0.6	9 ± 1.5	8 ± 0.7	9 ± 1.2	8 ± 1.7
	100	6 ± 1.5	6 ± 1.8	10 ± 1.5	8 ± 1.3	11 ± 1.2	10 ± 0.7
	333	7 ± 1.5	6 ± 1.5	13 ± 1.5	9 ± 1.7	9 ± 3.2	7 ± 0.7
	1,000	8 ± 1.5	5 ± 0.0	13 ± 3.8	6 ± 1.5	11 ± 3.4	7 ± 1.8
	3,333	5 ± 0.7	3 ± 2.0	12 ± 2.7	6 ± 0.9	11 ± 0.7	4 ± 0.9
	10,000	8 ± 3.8	1 ± 0.6	11 ± 3.0	7 ± 0.9	7 ± 0.7	7 ± 0.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		939 ± 81.5	784 ± 147.4	380 ± 34.1	141 ± 25.1	114 ± 11.0	161 ± 8.0
		–S9		**+10% hamster S9**		**+10% rat S9**	
		Trial 1	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
TA98							
	0	33 ± 5.6	18 ± 5.8	45 ± 3.5	36 ± 9.4	50 ± 4.0	32 ± 2.0
	100	23 ± 1.2	17 ± 1.2	49 ± 3.5	23 ± 0.7	41 ± 3.2	22 ± 2.6
	333	26 ± 2.5	15 ± 2.3	39 ± 1.8	29 ± 5.0	31 ± 1.3	19 ± 2.1
	1,000	24 ± 3.6	14 ± 1.2	39 ± 4.5	23 ± 2.2	39 ± 4.4	23 ± 4.7
	3,333	17 ± 2.3	17 ± 2.7	28 ± 5.2	19 ± 0.7	33 ± 2.9	18 ± 2.3
	10,000	11 ± 3.9	15 ± 2.1	17 ± 4.4	15 ± 3.3	20 ± 2.0	18 ± 4.4
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		362 ± 8.4	287 ± 9.1	703 ± 68.6	768 ± 56.4	1,410 ± 181.0	1,790 ± 71.2

[a] Study was performed at Case Western Reserve University. The detailed protocol and these data are presented by Zeiger *et al.* (1987).
[b] 0 µg/plate was the solvent control.
[c] Revertants are presented as mean ± standard error from three plates.
The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE C2
Frequency of Micronuclei in Peripheral Blood Normochromatic Erythrocytes of B6C3F$_1$ Mice Following Dermal Application of Pentaerythritol 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	1.20 ± 0.21		98.2 ± 0.1
Pentaerythritol triacrylate	0.75	10	0.75 ± 0.15	0.9253	98.1 ± 0.1
	1.5	10	1.00 ± 0.15	0.7269	98.4 ± 0.1
	3	10	0.90 ± 0.19	0.8229	98.3 ± 0.1
	6	10	1.05 ± 0.22	0.6727	98.4 ± 0.1
	12	10	1.05 ± 0.16	0.6727	98.2 ± 0.1
			P=0.394[e]		
Female					
Acetone		9	0.72 ± 0.15		98.2 ± 0.1
Pentaerythritol triacrylate	0.75	10	0.65 ± 0.15	0.6060	98.1 ± 0.1
	1.5	9	0.78 ± 0.19	0.4237	98.3 ± 0.1
	3	10	0.65 ± 0.17	0.6060	98.3 ± 0.1
	6	10	0.90 ± 0.18	0.2722	98.3 ± 0.1
	12	10	0.85 ± 0.11	0.3290	98.4 ± 0.1
			P=0.210		

[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 vehicle controls, significant at P≤0.005 (ILS, 1990)
[e] Vehicle control
Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed Cochran-Armitage trend test, significant at P≤0.025 (ILS, 1990)

TABLE C3
Frequency of Micronuclei in Peripheral Blood Normochromatic Erythrocytes of Tg.AC Hemizygous Mice Following Dermal Application of Pentaerythritol Triacrylate for 6 Months[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs (%)
Male					
Acetone[d]		12	0.58 ± 0.17		3.3
Pentaerythritol triacrylate	0.75	14	0.50 ± 0.14	0.6585	2.2
	1.5	15	0.63 ± 0.16	0.4076	2.1
	3	15	0.73 ± 0.16	0.2511	2.1
	6	12	0.96 ± 0.17	0.0694	11.7*
	12	10	1.15 ± 0.36	0.0206	8.1*
			P=0.001[e]		
Female					
Acetone		12	0.75 ± 0.21		2.2
Pentaerythritol triacrylate	0.75	14	0.61 ± 0.16	0.7344	2.0
	1.5	12	0.88 ± 0.21	0.3154	2.2
	3	12	1.04 ± 0.13	0.1428	2.5
	6	13	1.96 ± 0.31	0.0001	9.8*
	12	9	2.28 ± 0.49	0.0000	7.3*
			P≤0.001		

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

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

TABLE D1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Dermal Study of Pentaerythritol 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)	(4)	(2)	(2)	(2)	(10)
Hepatodiaphragmatic nodule	3 (30%)	4 (100%)	1 (50%)	2 (100%)	1 (50%)	3 (30%)
Tongue			(1)			
Cyst			1 (100%)			
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	2 (20%)					2 (20%)
Endocrine System						
None						
General Body System						
None						
Genital System						
Preputial gland	(10)					(10)
Inflammation, chronic active	1 (10%)					1 (10%)
Prostate	(10)					(10)
Inflammation, chronic active	1 (10%)					
Hematopoietic System						
Lymph node, mesenteric	(10)					(10)
Hyperplasia						1 (10%)
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Dermis, skin, site of application, inflammation, chronic active		1 (10%)	3 (30%)	10 (100%)	10 (100%)	9 (90%)
Epidermis, skin, site of application, degeneration		1 (10%)	6 (60%)	7 (70%)	7 (70%)	5 (50%)
Epidermis, skin, site of application, hyperplasia	1 (10%)	2 (20%)	7 (70%)	9 (90%)	10 (100%)	7 (70%)
Epidermis, skin, site of application, inflammation, suppurative					6 (60%)	3 (30%)
Epidermis, skin, site of application, necrosis			1 (10%)	1 (10%)	5 (50%)	2 (20%)
Sebaceous gland, skin, site of application, hyperplasia		2 (20%)	9 (90%)	9 (90%)	10 (100%)	9 (90%)
Skin, site of application, degeneration				1 (10%)		
Skin, site of application, hyperkeratosis	2 (20%)	8 (80%)	10 (100%)	10 (100%)	10 (100%)	9 (90%)

[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 Pentaerythritol 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	(10)					(10)
Inflammation, chronic active	7 (70%)					8 (80%)
Nose	(10)					(10)
Inflammation, acute	1 (10%)					
Special Senses System						
None						
Urinary System						
Kidney	(10)					(10)
Nephropathy	4 (40%)					6 (60%)

TABLE D2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 3-Month Dermal Study of Pentaerythritol 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)	(1)	(4)	(1)	(1)	(10)
Hepatodiaphragmatic nodule		1 (100%)	3 (75%)	1 (100%)	1 (100%)	2 (20%)
Inflammation, chronic active	9 (90%)					4 (40%)
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	2 (20%)					1 (10%)
Endocrine System						
Thyroid gland	(10)					(10)
Ultimobranchial cyst	1 (10%)					2 (20%)
General Body System						
None						
Genital System						
Clitoral gland	(10)				(1)	(10)
Atrophy						1 (10%)
Cyst	1 (10%)				1 (100%)	1 (10%)
Ovary	(10)	(1)			(1)	(10)
Cyst		1 (100%)			1 (100%)	
Uterus	(10)					(10)
Hydrometra	1 (10%)					5 (50%)
Hematopoietic System						
Lymph node, mesenteric	(10)					(10)
Hyperplasia						1 (10%)

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

TABLE D2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 3-Month Dermal Study of Pentaerythritol 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, fibrosis						1 (10%)
Dermis, skin, site of application, inflammation, chronic active			3 (30%)	4 (40%)	9 (90%)	10 (100%)
Epidermis, skin, site of application, degeneration			4 (40%)	5 (50%)	5 (50%)	6 (60%)
Epidermis, skin, site of application, hyperplasia			3 (30%)	8 (80%)	8 (80%)	9 (90%)
Epidermis, skin, site of application, inflammation, suppurative					4 (40%)	
Epidermis, skin, site of application, necrosis					3 (30%)	
Sebaceous gland, skin, site of application, hyperplasia			8 (80%)	8 (80%)	10 (100%)	9 (90%)
Skin, site of application, hyperkeratosis		4 (40%)	8 (80%)	9 (90%)	10 (100%)	10 (100%)
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Inflammation, chronic active	4 (40%)					7 (70%)
Special Senses System						
None						
Urinary System						
None						

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 3-Month Dermal Study of Pentaerythritol 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						1 (10%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(10)					(10)
Subcapsular, hyperplasia	1 (10%)					
General Body System						
None						
Genital System						
None						
Hematopoietic System						
None						
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Hyperkeratosis					1 (10%)	
Dermis, skin, site of application, fibrosis					6 (60%)	10 (100%)
Dermis, skin, site of application, inflammation, chronic active			8 (80%)	8 (80%)	10 (100%)	10 (100%)
Epidermis, hyperplasia					1 (10%)	
Epidermis, inflammation, suppurative					1 (10%)	
Epidermis, skin, site of application, degeneration			5 (50%)	5 (50%)	2 (20%)	4 (40%)
Epidermis, skin, site of application, hyperplasia			3 (30%)	8 (80%)	9 (90%)	10 (100%)
Epidermis, skin, site of application, inflammation, suppurative				2 (20%)		
Epidermis, skin, site of application, necrosis			2 (20%)	3 (30%)	7 (70%)	7 (70%)
Sebaceous gland, skin, site of application, hyperplasia			7 (70%)	9 (90%)	10 (100%)	10 (100%)
Skin, site of application, hyperkeratosis			5 (50%)	7 (70%)	8 (80%)	6 (60%)
Skin, site of application, necrosis					1 (10%)	

[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 Pentaerythritol 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						
Kidney	(10)					(10)
Nephropathy	1 (10%)					
Urinary bladder	(10)					(10)
Edema						1 (10%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 3-Month Dermal Study of Pentaerythritol 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
Early deaths						
Moribund	1					
Natural death			1			
Survivors						
Terminal sacrifice	9	10	9	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)		(1)			(10)
Hepatodiaphragmatic nodule			1 (100%)			
Inflammation, chronic active	1 (10%)					1 (10%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(10)					(10)
Accessory adrenal cortical nodule	1 (10%)					
Vacuolization cytoplasmic	6 (60%)					7 (70%)
Subcapsular, hyperplasia	8 (80%)					7 (70%)
General Body System						
None						
Genital System						
Ovary	(10)				(1)	(9)
Inflammation, chronic active					1 (100%)	1 (11%)
Uterus	(10)					(9)
Hydrometra						1 (11%)
Hematopoietic System						
None						
Integumentary System						
Skin	(9)	(10)	(10)	(10)	(10)	(10)
Dermis, skin, site of application, fibrosis					9 (90%)	10 (100%)
Dermis, skin, site of application, inflammation, chronic active		2 (20%)	5 (50%)	9 (90%)	9 (90%)	10 (100%)
Epidermis, skin, site of application, hyperplasia		1 (10%)	2 (20%)	6 (60%)	8 (80%)	10 (100%)
Epidermis, skin, site of application, necrosis					2 (20%)	3 (30%)
Sebaceous gland, skin, site of application, hyperplasia			9 (90%)	9 (90%)	8 (80%)	10 (100%)
Skin, site of application, hyperkeratosis		1 (10%)	2 (20%)	7 (70%)	7 (70%)	7 (70%)

[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 Pentaerythritol 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						
Kidney	(10)					(10)
Nephropathy	3 (30%)					
Urinary bladder	(10)					(10)
Edema	1 (10%)					2 (20%)

APPENDIX E
CLINICAL PATHOLOGY RESULTS

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Pentaerythritol 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	7	10	9	9	9
Day 23	8	9	10	9	10	8
Week 14	8	8	7	7	9	8
Hematocrit (%)						
Day 4	41.1 ± 0.4	42.5 ± 0.5	41.8 ± 0.4	41.2 ± 0.2	41.9 ± 0.4	41.8 ± 0.5
Day 23	42.5 ± 0.3	43.4 ± 0.7	42.9 ± 0.3	43.1 ± 0.3	43.8 ± 0.6	43.8 ± 0.6
Week 14	44.5 ± 0.4	44.9 ± 0.4	44.7 ± 0.3	44.8 ± 0.3	45.5 ± 0.4	46.2 ± 0.7
Hemoglobin (g/dL)						
Day 4	12.9 ± 0.1	13.3 ± 0.1	13.0 ± 0.1	12.9 ± 0.1	13.1 ± 0.1	13.0 ± 0.2
Day 23	13.8 ± 0.1	14.2 ± 0.2	13.9 ± 0.1	14.1 ± 0.1	14.1 ± 0.2	14.2 ± 0.1
Week 14	15.1 ± 0.1	15.2 ± 0.1	15.1 ± 0.1	15.2 ± 0.1	15.5 ± 0.1*	15.7 ± 0.2*
Erythrocytes (10^6/μL)						
Day 4	6.50 ± 0.08	6.77 ± 0.09	6.70 ± 0.07	6.60 ± 0.04	6.69 ± 0.08	6.68 ± 0.09
Day 23	7.41 ± 0.05	7.61 ± 0.12	7.53 ± 0.05	7.55 ± 0.06	7.67 ± 0.13	7.69 ± 0.10
Week 14	8.67 ± 0.07	8.79 ± 0.06	8.81 ± 0.05	8.73 ± 0.06	8.92 ± 0.06**	9.03 ± 0.14*
Reticulocytes (10^6/μL)						
Day 4	0.34 ± 0.02	0.38 ± 0.04	0.37 ± 0.04	0.39 ± 0.04	0.37 ± 0.03	0.35 ± 0.03
Day 23	0.16 ± 0.02	0.15 ± 0.01	0.14 ± 0.01	0.16 ± 0.02	0.16 ± 0.01	0.17 ± 0.01
Week 14	0.10 ± 0.01	0.12 ± 0.02	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Nucleated erythrocytes (10^3/μL)						
Day 4	0.03 ± 0.03	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.02 ± 0.02	0.03 ± 0.03	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	0.01 ± 0.01
Mean cell volume (fL)						
Day 4	63.3 ± 0.3	62.8 ± 0.3	62.3 ± 0.5	62.5 ± 0.3	62.7 ± 0.3	62.6 ± 0.3
Day 23	57.3 ± 0.2	57.1 ± 0.1	57.0 ± 0.2	57.1 ± 0.1	57.1 ± 0.2	56.9 ± 0.3
Week 14	51.4 ± 0.1	51.1 ± 0.1	50.7 ± 0.2	51.4 ± 0.4	51.0 ± 0.2	51.2 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.8 ± 0.2	19.7 ± 0.2	19.5 ± 0.1	19.6 ± 0.2	19.6 ± 0.1	19.5 ± 0.1
Day 23	18.7 ± 0.1	18.7 ± 0.1	18.5 ± 0.1	18.6 ± 0.1	18.5 ± 0.1	18.5 ± 0.1
Week 14	17.4 ± 0.1	17.3 ± 0.1	17.1 ± 0.0	17.4 ± 0.1	17.3 ± 0.1	17.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	31.3 ± 0.2	31.4 ± 0.2	31.2 ± 0.1	31.3 ± 0.2	31.3 ± 0.1	31.2 ± 0.1
Day 23	32.6 ± 0.2	32.8 ± 0.2	32.5 ± 0.1	32.6 ± 0.1	32.3 ± 0.2	32.5 ± 0.2
Week 14	33.8 ± 0.2	33.9 ± 0.1	33.8 ± 0.1	33.9 ± 0.2	34.0 ± 0.1	33.9 ± 0.1
Platelets (10^3/μL)						
Day 4	962.8 ± 21.9	876.7 ± 17.0	967.1 ± 22.7	955.9 ± 27.6	927.2 ± 20.0	984.0 ± 20.3
Day 23	826.3 ± 10.2	773.3 ± 10.7*	757.7 ± 26.1	802.3 ± 18.1	790.7 ± 12.3	785.0 ± 14.6
Week 14	734.1 ± 18.7	647.5 ± 20.2*	694.6 ± 40.3	662.0 ± 24.3	639.9 ± 24.5*	693.8 ± 9.7
Leukocytes (10^3/μL)						
Day 4	7.41 ± 0.32[b]	7.33 ± 0.33	7.49 ± 0.34	7.14 ± 0.22	7.34 ± 0.44	7.08 ± 0.50
Day 23	11.93 ± 0.29[b]	11.97 ± 0.47	11.28 ± 0.49	11.97 ± 0.61	11.34 ± 0.45	12.13 ± 0.59
Week 14	9.80 ± 0.58	9.98 ± 0.81	11.36 ± 0.51	9.59 ± 0.44	11.11 ± 0.54	10.74 ± 0.78
Segmented neutrophils (10^3/μL)						
Day 4	1.22 ± 0.12[b]	1.00 ± 0.12	1.25 ± 0.11	1.20 ± 0.09	1.21 ± 0.11	1.51 ± 0.15
Day 23	1.01 ± 0.06[b]	1.02 ± 0.11	1.02 ± 0.07	1.00 ± 0.10	1.28 ± 0.09	2.07 ± 0.20**
Week 14	1.67 ± 0.26	2.12 ± 0.34	2.39 ± 0.29	1.51 ± 0.17	2.66 ± 0.25*	2.52 ± 0.27

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Pentaerythritol 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	7	10	9	9	9
Day 23	8	9	10	9	10	8
Week 14	8	8	7	7	9	8
Bands (10^3/μL)						
Day 4	0.01 ± 0.01[b]	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Day 23	0.00 ± 0.00[b]	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	6.09 ± 0.30[b]	6.10 ± 0.36	6.03 ± 0.27	5.70 ± 0.28	5.89 ± 0.38	5.35 ± 0.45
Day 23	10.73 ± 0.25[b]	10.84 ± 0.44	10.08 ± 0.44	10.82 ± 0.54	9.92 ± 0.46	9.90 ± 0.54
Week 14	7.88 ± 0.38	7.57 ± 0.47	8.71 ± 0.47	7.86 ± 0.42	8.18 ± 0.48	7.89 ± 0.61
Monocytes (10^3/μL)						
Day 4	0.10 ± 0.01[b]	0.21 ± 0.04	0.19 ± 0.05	0.20 ± 0.04	0.19 ± 0.03	0.20 ± 0.05
Day 23	0.09 ± 0.03[b]	0.09 ± 0.03	0.09 ± 0.03	0.13 ± 0.03	0.04 ± 0.02	0.11 ± 0.03
Week 14	0.18 ± 0.03	0.21 ± 0.01	0.18 ± 0.03	0.15 ± 0.04	0.22 ± 0.05	0.23 ± 0.04
Basophils (10^3/μL)						
Day 4	0.000 ± 0.000[b]	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[b]	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	0.00 ± 0.00[b]	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.02	0.05 ± 0.02*	0.01 ± 0.01
Day 23	0.10 ± 0.03[b]	0.03 ± 0.02	0.08 ± 0.02	0.03 ± 0.02	0.09 ± 0.03	0.03 ± 0.02
Week 14	0.06 ± 0.03	0.08 ± 0.04	0.08 ± 0.03	0.06 ± 0.02	0.05 ± 0.02	0.10 ± 0.02
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	10.0 ± 0.4	10.8 ± 0.6	10.9 ± 0.5	9.3 ± 0.4	9.7 ± 0.5	9.5 ± 0.3
Day 23	11.6 ± 0.3	12.4 ± 0.6	10.8 ± 0.4	11.0 ± 0.4	11.5 ± 0.3	11.3 ± 0.3
Week 14	16.8 ± 0.5	16.8 ± 0.7	18.2 ± 0.4	16.7 ± 0.4	16.1 ± 0.5	17.5 ± 1.8
Creatinine (mg/dL)						
Day 4	0.45 ± 0.03	0.44 ± 0.02	0.36 ± 0.04	0.48 ± 0.05	0.44 ± 0.02	0.47 ± 0.06
Day 23	0.43 ± 0.02	0.43 ± 0.02	0.42 ± 0.01	0.43 ± 0.02	0.42 ± 0.01	0.43 ± 0.02
Week 14	0.56 ± 0.02	0.59 ± 0.01	0.58 ± 0.01	0.58 ± 0.01	0.58 ± 0.01	0.56 ± 0.02
Total protein (g/dL)						
Day 4	5.4 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.4 ± 0.1
Day 23	5.7 ± 0.1	5.7 ± 0.0	5.6 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.8 ± 0.1
Week 14	6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.0	6.8 ± 0.1	6.9 ± 0.1	6.8 ± 0.1
Albumin (g/dL)						
Day 4	4.0 ± 0.0	4.2 ± 0.1	4.1 ± 0.0	4.1 ± 0.0	4.2 ± 0.0*	4.1 ± 0.1
Day 23	4.1 ± 0.1	4.2 ± 0.0	4.1 ± 0.0	4.2 ± 0.1	4.2 ± 0.1	4.3 ± 0.0
Week 14	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.0	4.6 ± 0.1	4.7 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	98 ± 3	92 ± 5	96 ± 3	100 ± 4	99 ± 3	88 ± 4
Day 23	71 ± 3	72 ± 2	65 ± 2	66 ± 2	70 ± 1	71 ± 2
Week 14	92 ± 5	114 ± 16	99 ± 5	90 ± 3	93 ± 3	104 ± 5

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Pentaerythritol 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	827 ± 20	878 ± 25	833 ± 13	827 ± 28	881 ± 36	822 ± 23
Day 23	518 ± 14	523 ± 8	502 ± 10	508 ± 10	506 ± 13	526 ± 10
Week 14	294 ± 6	302 ± 6	316 ± 5	306 ± 7	289 ± 7	323 ± 6*
Creatine kinase (IU/L)						
Day 4	403 ± 72	440 ± 69	436 ± 99	412 ± 84	296 ± 47[c]	642 ± 106
Day 23	398 ± 139	338 ± 44	241 ± 45	192 ± 27	205 ± 24	287 ± 52
Week 14	177 ± 28	175 ± 18	242 ± 64	242 ± 44	201 ± 23	166 ± 25[c]
Sorbitol dehydrogenase (IU/L)						
Day 4	17 ± 1	17 ± 1	18 ± 1	18 ± 1	18 ± 1	17 ± 1
Day 23	17 ± 1	18 ± 1	16 ± 1	16 ± 1	16 ± 1	15 ± 1
Week 14	29 ± 1	43 ± 9	29 ± 2	27 ± 2	25 ± 2	29 ± 3
Bile acids (µmol/L)						
Day 4	23.1 ± 2.6	19.5 ± 0.7	20.5 ± 1.4	27.1 ± 4.1	25.7 ± 2.7	27.5 ± 3.4
Day 23	30.7 ± 3.5	32.5 ± 3.2	30.4 ± 2.8	28.4 ± 2.2	29.6 ± 3.2	31.9 ± 3.0
Week 14	26.2 ± 2.5	23.2 ± 4.5	23.5 ± 2.7	22.0 ± 1.9	27.8 ± 4.2	26.5 ± 3.1

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Pentaerythritol 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	9	10	10	8	9	8
Day 23	9	10	9	9	10	10
Week 14	8	9	8	6	8	9
Hematocrit (%)						
Day 4	42.9 ± 0.4	43.2 ± 0.5	43.0 ± 0.5	42.8 ± 0.5	42.3 ± 0.3	42.1 ± 0.6
Day 23	46.0 ± 0.5	46.5 ± 0.4	47.1 ± 0.7	46.4 ± 0.4	46.1 ± 0.4	46.4 ± 0.5
Week 14	43.9 ± 0.5	44.9 ± 0.6	45.2 ± 0.5	44.4 ± 0.4	43.8 ± 0.4	44.6 ± 0.3
Hemoglobin (g/dL)						
Day 4	13.9 ± 0.1	14.1 ± 0.1	14.1 ± 0.2	14.0 ± 0.1	13.9 ± 0.1	13.9 ± 0.2
Day 23	14.8 ± 0.2	15.1 ± 0.1	15.3 ± 0.2	15.0 ± 0.1	14.9 ± 0.1	15.0 ± 0.2
Week 14	14.9 ± 0.1	15.1 ± 0.1	15.3 ± 0.2*	15.3 ± 0.1	15.0 ± 0.1	15.2 ± 0.1
Erythrocytes (10^6/µL)						
Day 4	7.25 ± 0.07	7.29 ± 0.10	7.27 ± 0.10	7.24 ± 0.08	7.17 ± 0.04	7.19 ± 0.11
Day 23	7.96 ± 0.10	8.09 ± 0.08	8.19 ± 0.13	8.03 ± 0.07	7.98 ± 0.08	8.07 ± 0.11
Week 14	8.04 ± 0.07	8.21 ± 0.08	8.31 ± 0.09	8.22 ± 0.11	8.13 ± 0.07	8.21 ± 0.08
Reticulocytes (10^6/µL)						
Day 4	0.16 ± 0.03	0.23 ± 0.03	0.19 ± 0.03	0.21 ± 0.03	0.20 ± 0.02	0.18 ± 0.03
Day 23	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01
Week 14	0.11 ± 0.01	0.09 ± 0.01	0.11 ± 0.02	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.02
Nucleated erythrocytes (10^3/µL)						
Day 4	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.00 ± 0.00	0.04 ± 0.04	0.04 ± 0.02
Mean cell volume (fL)						
Day 4	59.1 ± 0.2	59.2 ± 0.2	59.2 ± 0.2	59.0 ± 0.1	59.0 ± 0.2	58.6 ± 0.4
Day 23	57.8 ± 0.2	57.5 ± 0.1	57.6 ± 0.2	57.8 ± 0.1	57.7 ± 0.1	57.6 ± 0.2
Week 14	54.6 ± 0.2	54.7 ± 0.3	54.4 ± 0.1	54.0 ± 0.4	53.9 ± 0.3	54.3 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.2 ± 0.1	19.4 ± 0.1	19.3 ± 0.1	19.3 ± 0.1	19.3 ± 0.1	19.3 ± 0.1
Day 23	18.6 ± 0.1	18.7 ± 0.1	18.6 ± 0.1	18.7 ± 0.0	18.6 ± 0.1	18.6 ± 0.1
Week 14	18.6 ± 0.1	18.4 ± 0.1	18.5 ± 0.1	18.6 ± 0.1	18.5 ± 0.1	18.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.5 ± 0.2	32.7 ± 0.2	32.7 ± 0.1	32.7 ± 0.1	32.8 ± 0.1	33.0 ± 0.2
Day 23	32.3 ± 0.2	32.6 ± 0.1	32.4 ± 0.1	32.3 ± 0.1	32.3 ± 0.1	32.4 ± 0.2
Week 14	34.0 ± 0.3	33.6 ± 0.2	34.0 ± 0.2	34.4 ± 0.2	34.3 ± 0.2	34.1 ± 0.1
Platelets (10^3/µL)						
Day 4	815.2 ± 21.9	791.6 ± 33.8	783.3 ± 35.3	802.6 ± 38.4	838.4 ± 22.0	808.5 ± 51.4
Day 23	732.1 ± 29.5	639.3 ± 42.4	689.0 ± 31.9	737.6 ± 6.9	701.2 ± 27.4	704.0 ± 22.4
Week 14	619.9 ± 31.2	588.6 ± 22.8	638.6 ± 44.5	582.5 ± 38.4	653.6 ± 26.4	644.3 ± 30.2
Leukocytes (10^3/µL)						
Day 4	9.50 ± 0.38	10.05 ± 0.34	9.39 ± 0.18	9.23 ± 0.32	8.99 ± 0.41	9.16 ± 0.43
Day 23	11.70 ± 0.57	12.01 ± 0.63	12.63 ± 0.34	11.48 ± 0.51	11.52 ± 0.60	13.13 ± 0.50
Week 14	10.89 ± 0.75	10.51 ± 0.56	11.74 ± 0.94	10.83 ± 0.54	9.19 ± 0.71	11.19 ± 0.75
Segmented neutrophils (10^3/µL)						
Day 4	1.26 ± 0.14	1.21 ± 0.11	1.12 ± 0.09	1.16 ± 0.13	1.15 ± 0.14	1.59 ± 0.09
Day 23	1.03 ± 0.13	1.23 ± 0.12	1.13 ± 0.09	1.07 ± 0.10	0.99 ± 0.12	2.01 ± 0.14**
Week 14	1.84 ± 0.32	1.75 ± 0.22	1.88 ± 0.25	1.67 ± 0.18	2.06 ± 0.34	2.22 ± 0.29

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Pentaerythritol 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	9	10	10	8	9	8
Day 23	9	10	9	9	10	10
Week 14	8	9	8	6	8	9
Bands (10^3/µL)						
Day 4	0.03 ± 0.02	0.03 ± 0.03	0.06 ± 0.03	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
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	7.79 ± 0.35	8.56 ± 0.24	7.87 ± 0.17	7.75 ± 0.28	7.43 ± 0.35	7.22 ± 0.37
Day 23	10.34 ± 0.58	10.53 ± 0.56	11.34 ± 0.37	10.17 ± 0.53	10.23 ± 0.52	10.80 ± 0.45
Week 14	8.56 ± 0.46	8.39 ± 0.52	9.21 ± 0.83	8.80 ± 0.50	6.78 ± 0.51	8.42 ± 0.58
Monocytes (10^3/µL)						
Day 4	0.39 ± 0.07	0.23 ± 0.06	0.31 ± 0.03	0.27 ± 0.07	0.37 ± 0.07	0.28 ± 0.05
Day 23	0.18 ± 0.02	0.12 ± 0.03	0.10 ± 0.02	0.15 ± 0.03	0.21 ± 0.03	0.17 ± 0.04
Week 14	0.37 ± 0.08	0.30 ± 0.09	0.49 ± 0.09	0.28 ± 0.11	0.25 ± 0.04	0.43 ± 0.05
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	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.03 ± 0.02	0.06 ± 0.02
Day 23	0.15 ± 0.04	0.12 ± 0.04	0.07 ± 0.02	0.09 ± 0.04	0.09 ± 0.03	0.15 ± 0.04
Week 14	0.12 ± 0.03	0.07 ± 0.03	0.16 ± 0.05	0.08 ± 0.03	0.09 ± 0.03	0.13 ± 0.03
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	12.6 ± 0.6	12.1 ± 0.6	11.2 ± 0.5	11.4 ± 0.4	10.9 ± 0.4	11.5 ± 0.4
Day 23	15.7 ± 0.5	15.5 ± 0.4	14.9 ± 0.7	14.9 ± 0.5	15.3 ± 0.4	14.4 ± 0.4
Week 14	17.8 ± 0.4	17.9 ± 0.6	16.8 ± 0.6	16.8 ± 0.6	16.3 ± 0.6	17.0 ± 0.6
Creatinine (mg/dL)						
Day 4	0.40 ± 0.02	0.40 ± 0.00	0.41 ± 0.01	0.40 ± 0.00	0.41 ± 0.01	0.40 ± 0.02
Day 23	0.40 ± 0.00	0.40 ± 0.00	0.38 ± 0.01	0.39 ± 0.01	0.40 ± 0.00	0.38 ± 0.01
Week 14	0.51 ± 0.01	0.50 ± 0.00	0.51 ± 0.01	0.51 ± 0.02	0.49 ± 0.02	0.51 ± 0.01
Total protein (g/dL)						
Day 4	5.6 ± 0.0	5.7 ± 0.1	5.6 ± 0.1	5.7 ± 0.0	5.6 ± 0.0	5.7 ± 0.1
Day 23	5.6 ± 0.1	5.4 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.6 ± 0.1
Week 14	6.4 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.4 ± 0.1
Albumin (g/dL)						
Day 4	4.3 ± 0.0	4.4 ± 0.0	4.3 ± 0.0	4.4 ± 0.0	4.3 ± 0.0	4.4 ± 0.1
Day 23	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.3 ± 0.0
Week 14	4.5 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	70 ± 2	68 ± 2	77 ± 2	76 ± 2	71 ± 2	76 ± 1
Day 23	63 ± 2	64 ± 1	67 ± 1	62 ± 2	65 ± 2	71 ± 2
Week 14	90 ± 3	101 ± 7	108 ± 9	96 ± 5	95 ± 5	92 ± 4

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Pentaerythritol 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	629 ± 10	626 ± 12	627 ± 13	619 ± 12	638 ± 15	625 ± 15
Day 23	445 ± 9	440 ± 9	462 ± 10	461 ± 9	429 ± 6	440 ± 5
Week 14	292 ± 4	310 ± 6	301 ± 8	306 ± 11	294 ± 6	316 ± 7
Creatine kinase (IU/L)						
Day 4	511 ± 84	449 ± 68	361 ± 41	649 ± 163	531 ± 101	761 ± 118
Day 23	380 ± 60	281 ± 29[c]	457 ± 94	221 ± 26[c]	278 ± 42[c]	250 ± 47[c]
Week 14	248 ± 29	337 ± 87	427 ± 70	275 ± 49	293 ± 53	252 ± 50
Sorbitol dehydrogenase (IU/L)						
Day 4	19 ± 0	19 ± 1	18 ± 0	18 ± 0	18 ± 0	19 ± 1
Day 23	18 ± 1	19 ± 1	18 ± 1	17 ± 1	17 ± 1	15 ± 1
Week 14	23 ± 1	25 ± 2	27 ± 2	24 ± 2	22 ± 1	21 ± 1
Bile acids (μmol/L)						
Day 4	18.5 ± 1.5	19.1 ± 0.9	21.1 ± 1.1	21.9 ± 1.3	22.6 ± 2.3	20.9 ± 1.7
Day 23	25.3 ± 3.0	25.2 ± 2.0	22.1 ± 2.8	25.9 ± 2.6	21.4 ± 1.6	23.4 ± 2.9
Week 14	30.7 ± 5.1	23.2 ± 1.3	30.7 ± 2.6	23.5 ± 2.2	28.3 ± 3.7	28.6 ± 3.2

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a]
[b] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[c] n=7
n=9

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

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
n	10	10	10	10	10	10
Hematocrit (%)	46.0 ± 0.4	45.7 ± 0.4	45.3 ± 0.7	45.1 ± 0.6	44.2 ± 0.3**	43.3 ± 0.3**
Hemoglobin (g/dL)	16.0 ± 0.1	16.0 ± 0.1	15.8 ± 0.2	15.6 ± 0.1*	15.5 ± 0.1**	15.1 ± 0.1**
Erythrocytes (10^6/μL)	10.02 ± 0.09	9.97 ± 0.11	9.85 ± 0.15	9.82 ± 0.11	9.62 ± 0.08**	9.51 ± 0.09**
Reticulocytes (10^6/μL)	0.05 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.05 ± 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)	46.0 ± 0.2	45.9 ± 0.1	46.0 ± 0.2	45.9 ± 0.2	45.9 ± 0.1	45.6 ± 0.2
Mean cell hemoglobin (pg)	16.0 ± 0.1	16.1 ± 0.0	16.1 ± 0.1	15.8 ± 0.1	16.1 ± 0.1	15.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.8 ± 0.1	35.0 ± 0.1	35.0 ± 0.2	34.5 ± 0.1	35.0 ± 0.1	34.9 ± 0.1
Platelets (10^3/μL)	772.3 ± 35.6	816.3 ± 37.5	845.5 ± 31.9	842.1 ± 26.7	902.5 ± 28.4**	947.7 ± 30.0**
Leukocytes (10^3/μL)	3.72 ± 0.49	3.54 ± 0.54	3.08 ± 0.39	3.39 ± 0.33	4.43 ± 0.22	5.98 ± 1.13
Segmented neutrophils (10^3/μL)	0.62 ± 0.07	0.64 ± 0.15	0.50 ± 0.07	0.57 ± 0.07	1.14 ± 0.12*	3.31 ± 0.98**
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)	2.98 ± 0.44	2.80 ± 0.42	2.49 ± 0.35	2.71 ± 0.27	3.17 ± 0.19	2.54 ± 0.20
Monocytes (10^3/μL)	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.03
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)	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.02
Female						
n	9	10	9	10	10	10
Hematocrit (%)	46.5 ± 0.3	46.8 ± 0.8	46.5 ± 0.7	46.2 ± 0.3	44.6 ± 0.3**	44.2 ± 0.3**
Hemoglobin (g/dL)	16.3 ± 0.1	16.3 ± 0.2	16.3 ± 0.2	16.0 ± 0.1	15.6 ± 0.1**	15.4 ± 0.1**
Erythrocytes (10^6/μL)	10.17 ± 0.08	10.07 ± 0.19	10.03 ± 0.18	9.94 ± 0.08	9.58 ± 0.08**	9.65 ± 0.08**
Reticulocytes (10^6/μL)	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	45.8 ± 0.1	46.4 ± 0.1**	46.3 ± 0.2	46.5 ± 0.2**	46.6 ± 0.1**	45.8 ± 0.3
Mean cell hemoglobin (pg)	16.0 ± 0.1	16.1 ± 0.1	16.2 ± 0.1	16.1 ± 0.1	16.2 ± 0.1	16.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	35.0 ± 0.1	34.8 ± 0.2	34.9 ± 0.1	34.7 ± 0.2	34.9 ± 0.1	34.9 ± 0.1
Platelets (10^3/μL)	655.3 ± 29.4	652.7 ± 47.4	592.8 ± 38.4	722.9 ± 36.7	730.2 ± 18.0	798.2 ± 22.3**
Leukocytes (10^3/μL)	4.60 ± 0.29	4.50 ± 0.31	4.34 ± 0.26	4.57 ± 0.30	4.63 ± 0.42	6.35 ± 0.54*
Segmented neutrophils (10^3/μL)	0.57 ± 0.08	0.43 ± 0.05	0.46 ± 0.05	0.54 ± 0.06	0.63 ± 0.08	1.37 ± 0.32**
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)	3.92 ± 0.21	3.98 ± 0.27	3.81 ± 0.25	3.94 ± 0.26	3.91 ± 0.36	4.86 ± 0.26
Monocytes (10^3/μL)	0.08 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.09 ± 0.02
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)	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01

* 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.

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 Pentaerythritol 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	182 ± 5	176 ± 1	174 ± 2	171 ± 4*	173 ± 2*	157 ± 2**
Heart						
Absolute	0.690 ± 0.013	0.679 ± 0.024	0.678 ± 0.014	0.677 ± 0.018	0.739 ± 0.055	0.714 ± 0.015
Relative	3.791 ± 0.055	3.853 ± 0.130	3.892 ± 0.067	3.967 ± 0.099	4.272 ± 0.289*	4.555 ± 0.147**
R. Kidney						
Absolute	0.741 ± 0.023	0.738 ± 0.016	0.723 ± 0.013	0.734 ± 0.019	0.753 ± 0.023	0.712 ± 0.026
Relative	4.075 ± 0.142	4.189 ± 0.075	4.151 ± 0.043	4.299 ± 0.072	4.363 ± 0.122	4.529 ± 0.110**
Liver						
Absolute	9.055 ± 0.256	8.454 ± 0.170	8.583 ± 0.302	8.333 ± 0.268	8.789 ± 0.233	7.644 ± 0.159**
Relative	49.8 ± 1.2	48.0 ± 0.8	49.3 ± 1.4	48.8 ± 1.0	50.9 ± 1.3	48.7 ± 0.7
Lung						
Absolute	1.365 ± 0.062	1.342 ± 0.053	1.239 ± 0.054	1.280 ± 0.050	1.136 ± 0.044**	1.105 ± 0.046**
Relative	7.514 ± 0.392	7.620 ± 0.305	7.121 ± 0.339	7.509 ± 0.312	6.573 ± 0.209	7.046 ± 0.326
R. Testis						
Absolute	1.129 ± 0.029	1.155 ± 0.012	1.111 ± 0.016	1.147 ± 0.005	1.133 ± 0.008	1.128 ± 0.014
Relative	6.211 ± 0.174	6.559 ± 0.083	6.382 ± 0.072	6.735 ± 0.152*	6.567 ± 0.038*	7.186 ± 0.096**
Thymus						
Absolute	0.410 ± 0.010	0.374 ± 0.035	0.399 ± 0.015	0.338 ± 0.031	0.377 ± 0.029	0.340 ± 0.015
Relative	2.251 ± 0.025	2.120 ± 0.192	2.289 ± 0.084	1.978 ± 0.183	2.180 ± 0.155	2.164 ± 0.096
Female						
Necropsy body wt	125 ± 2	125 ± 2	124 ± 2	124 ± 4	119 ± 1	114 ± 3**
Heart						
Absolute	0.527 ± 0.018	0.485 ± 0.042	0.503 ± 0.010	0.525 ± 0.017	0.525 ± 0.008	0.531 ± 0.011
Relative	4.227 ± 0.101	3.889 ± 0.330	4.076 ± 0.090	4.231 ± 0.071	4.415 ± 0.036	4.659 ± 0.052
R. Kidney						
Absolute	0.535 ± 0.008	0.550 ± 0.012	0.553 ± 0.010	0.580 ± 0.018	0.571 ± 0.009	0.566 ± 0.013
Relative	4.288 ± 0.051	4.405 ± 0.059	4.479 ± 0.056	4.673 ± 0.095**	4.796 ± 0.061**	4.967 ± 0.045**
Liver						
Absolute	5.621 ± 0.156	5.810 ± 0.135	5.861 ± 0.137	5.857 ± 0.157	5.721 ± 0.082	5.655 ± 0.241
Relative	45.1 ± 1.0	46.5 ± 1.0	47.5 ± 0.6	47.2 ± 0.8	48.1 ± 0.6*	49.5 ± 0.9**
Lung						
Absolute	0.911 ± 0.058	1.114 ± 0.098	0.935 ± 0.035	1.078 ± 0.036	0.887 ± 0.027	0.903 ± 0.055
Relative	7.298 ± 0.419	8.898 ± 0.714*	7.590 ± 0.331	8.678 ± 0.163	7.460 ± 0.255	7.904 ± 0.351
Thymus						
Absolute	0.336 ± 0.025	0.373 ± 0.035	0.332 ± 0.015	0.326 ± 0.010	0.295 ± 0.015	0.320 ± 0.009
Relative	2.696 ± 0.209	2.985 ± 0.256	2.688 ± 0.115	2.635 ± 0.125	2.481 ± 0.142	2.811 ± 0.053

* 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 Pentaerythritol 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	288 ± 11	283 ± 7	288 ± 6	275 ± 7	266 ± 5*	259 ± 5**
Heart						
Absolute	0.905 ± 0.030	0.933 ± 0.026	0.923 ± 0.024	0.863 ± 0.016	0.900 ± 0.021	0.882 ± 0.019
Relative	3.154 ± 0.056	3.301 ± 0.069	3.213 ± 0.075	3.142 ± 0.030	3.393 ± 0.071**	3.406 ± 0.053**
R. Kidney						
Absolute	1.000 ± 0.030	0.956 ± 0.025	0.997 ± 0.023	0.972 ± 0.022	0.985 ± 0.015	0.974 ± 0.018
Relative	3.489 ± 0.068	3.381 ± 0.056	3.469 ± 0.048	3.538 ± 0.047	3.716 ± 0.067**	3.762 ± 0.046**
Liver						
Absolute	11.501 ± 0.571	11.260 ± 0.401	11.678 ± 0.435	11.269 ± 0.462	10.525 ± 0.310	10.448 ± 0.275
Relative	39.9 ± 0.8	39.8 ± 0.9	40.5 ± 0.9	40.9 ± 0.9	39.6 ± 0.8	40.3 ± 0.5
Lung						
Absolute	1.726 ± 0.079	1.723 ± 0.094	1.821 ± 0.079	1.675 ± 0.059	1.761 ± 0.090	1.715 ± 0.062
Relative	6.041 ± 0.301	6.097 ± 0.312	6.324 ± 0.209	6.097 ± 0.187	6.610 ± 0.261	6.639 ± 0.270
R. Testis						
Absolute	1.381 ± 0.032	1.386 ± 0.022	1.399 ± 0.016	1.361 ± 0.018	1.371 ± 0.016	1.386 ± 0.020
Relative	4.828 ± 0.094	4.913 ± 0.092	4.878 ± 0.062	4.965 ± 0.078	5.175 ± 0.091**	5.359 ± 0.092**
Thymus						
Absolute	0.362 ± 0.019	0.343 ± 0.010	0.336 ± 0.008	0.314 ± 0.011**	0.289 ± 0.010**	0.290 ± 0.012**
Relative	1.256 ± 0.041	1.216 ± 0.042	1.175 ± 0.041	1.141 ± 0.028*	1.089 ± 0.032**	1.119 ± 0.044**
Female						
Necropsy body wt	174 ± 4	174 ± 3	172 ± 4	171 ± 3	167 ± 4	166 ± 4
Heart						
Absolute	0.663 ± 0.011	0.692 ± 0.017	0.678 ± 0.019	0.683 ± 0.022	0.669 ± 0.024	0.652 ± 0.017
Relative	3.816 ± 0.068	3.981 ± 0.053	3.960 ± 0.118	3.993 ± 0.123	4.009 ± 0.145	3.929 ± 0.068
R. Kidney						
Absolute	0.686 ± 0.011	0.664 ± 0.011	0.652 ± 0.014	0.681 ± 0.013	0.678 ± 0.014	0.686 ± 0.018
Relative	3.945 ± 0.045	3.826 ± 0.057	3.800 ± 0.045	3.979 ± 0.053	4.057 ± 0.046	4.124 ± 0.030*
Liver						
Absolute	6.698 ± 0.245	6.655 ± 0.153	6.175 ± 0.236	6.398 ± 0.107	6.293 ± 0.254	6.332 ± 0.243
Relative	38.4 ± 0.9	38.3 ± 0.5	35.9 ± 0.7*	37.4 ± 0.5	37.5 ± 0.7	38.0 ± 0.6
Lung						
Absolute	1.304 ± 0.077	1.295 ± 0.062	1.311 ± 0.076	1.309 ± 0.050	1.241 ± 0.072	1.365 ± 0.077
Relative	7.469 ± 0.361	7.432 ± 0.273	7.609 ± 0.349	7.658 ± 0.285	7.419 ± 0.408	8.174 ± 0.310
Thymus						
Absolute	0.287 ± 0.012	0.266 ± 0.011	0.264 ± 0.013	0.253 ± 0.014	0.253 ± 0.011	0.253 ± 0.010
Relative	1.654 ± 0.076	1.533 ± 0.055	1.537 ± 0.077	1.471 ± 0.064	1.513 ± 0.061	1.527 ± 0.070

* 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 F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 2-Week Dermal Study of Pentaerythritol 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	24.6 ± 0.4	25.3 ± 0.3	26.1 ± 0.2**	25.4 ± 0.4	24.3 ± 0.3	24.2 ± 0.3
Heart						
Absolute	0.133 ± 0.003	0.129 ± 0.003	0.148 ± 0.004*	0.156 ± 0.001**	0.135 ± 0.005	0.142 ± 0.006
Relative	5.418 ± 0.074	5.075 ± 0.078	5.670 ± 0.164	6.141 ± 0.084**	5.535 ± 0.152	5.877 ± 0.233
R. Kidney						
Absolute	0.238 ± 0.007	0.237 ± 0.005	0.256 ± 0.004	0.255 ± 0.013	0.234 ± 0.007	0.231 ± 0.009
Relative	9.684 ± 0.163	9.337 ± 0.143	9.801 ± 0.133	10.002 ± 0.385	9.614 ± 0.193	9.540 ± 0.374
Liver						
Absolute	1.461 ± 0.066	1.472 ± 0.035	1.549 ± 0.032	1.556 ± 0.053	1.409 ± 0.058	1.351 ± 0.021
Relative	59.4 ± 2.1	58.1 ± 1.5	59.3 ± 0.8	61.3 ± 2.2	57.9 ± 1.7	55.8 ± 0.3
Lung						
Absolute	0.196 ± 0.011	0.193 ± 0.005	0.203 ± 0.007	0.184 ± 0.004	0.181 ± 0.008	0.184 ± 0.010
Relative	7.997 ± 0.458	7.640 ± 0.308	7.777 ± 0.285	7.232 ± 0.141	7.442 ± 0.255	7.622 ± 0.458
R. Testis						
Absolute	0.100 ± 0.002	0.094 ± 0.002	0.100 ± 0.003	0.097 ± 0.004	0.101 ± 0.002	0.098 ± 0.003
Relative	4.062 ± 0.070	3.709 ± 0.052*	3.820 ± 0.082	3.830 ± 0.117	4.139 ± 0.060	4.066 ± 0.123
Thymus						
Absolute	0.056 ± 0.003	0.053 ± 0.003	0.051 ± 0.004	0.041 ± 0.004*	0.034 ± 0.004**	0.030 ± 0.004**
Relative	2.277 ± 0.130	2.113 ± 0.141	1.940 ± 0.149	1.615 ± 0.158**	1.377 ± 0.156**	1.253 ± 0.166**
Female						
Necropsy body wt	20.9 ± 0.6	21.2 ± 0.4	21.9 ± 0.7	19.6 ± 1.3	22.1 ± 0.6	21.2 ± 0.5
Heart						
Absolute	0.123 ± 0.004	0.126 ± 0.004	0.120 ± 0.005	0.113 ± 0.005	0.129 ± 0.008	0.123 ± 0.006
Relative	5.865 ± 0.142	5.969 ± 0.204	5.480 ± 0.252	5.847 ± 0.251	5.815 ± 0.204	5.784 ± 0.148
R. Kidney						
Absolute	0.172 ± 0.006	0.171 ± 0.004	0.176 ± 0.004	0.175 ± 0.006	0.188 ± 0.006	0.189 ± 0.005
Relative	8.239 ± 0.176	8.110 ± 0.257	8.064 ± 0.135	9.046 ± 0.390	8.528 ± 0.251	8.901 ± 0.137
Liver						
Absolute	1.268 ± 0.060	1.227 ± 0.019	1.339 ± 0.050	1.180 ± 0.106	1.386 ± 0.053	1.404 ± 0.155
Relative	60.5 ± 1.7	58.0 ± 0.5	61.3 ± 1.7	60.0 ± 2.4	62.6 ± 1.2	65.8 ± 6.0
Lung						
Absolute	0.188 ± 0.006	0.164 ± 0.007	0.165 ± 0.005	0.170 ± 0.017	0.210 ± 0.023[b]	0.177 ± 0.007
Relative	9.010 ± 0.193	7.782 ± 0.411	7.544 ± 0.273	8.713 ± 0.720	9.460 ± 0.733[b]	8.362 ± 0.411
Thymus						
Absolute	0.076 ± 0.002	0.077 ± 0.005	0.066 ± 0.014	0.059 ± 0.006	0.058 ± 0.008	0.053 ± 0.002*
Relative	3.670 ± 0.180	3.674 ± 0.293	2.975 ± 0.614	3.004 ± 0.289	2.621 ± 0.330*	2.506 ± 0.038*

* Significantly different (P≤0.05) from the vehicle control group by Williams' or Dunnett's test
** P≤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=4

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

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	33.8 ± 0.5	36.2 ± 0.8	35.1 ± 0.8	33.7 ± 1.1	32.8 ± 0.4	33.3 ± 0.5
Heart						
Absolute	0.167 ± 0.003	0.177 ± 0.006	0.182 ± 0.007	0.174 ± 0.007	0.179 ± 0.008	0.173 ± 0.003
Relative	4.933 ± 0.104	4.881 ± 0.129	5.170 ± 0.135	5.163 ± 0.117	5.461 ± 0.221*	5.206 ± 0.095
R. Kidney						
Absolute	0.323 ± 0.007	0.355 ± 0.010	0.353 ± 0.013	0.328 ± 0.010	0.324 ± 0.009	0.324 ± 0.010
Relative	9.556 ± 0.209	9.841 ± 0.291	10.062 ± 0.272	9.779 ± 0.268	9.885 ± 0.208	9.702 ± 0.205
Liver						
Absolute	1.692 ± 0.036	1.818 ± 0.048	1.745 ± 0.046	1.680 ± 0.055	1.677 ± 0.049	1.640 ± 0.044
Relative	50.1 ± 1.0	50.3 ± 1.0	49.7 ± 0.7	50.0 ± 0.8	51.1 ± 1.1	49.2 ± 1.0
Lung						
Absolute	0.283 ± 0.015	0.265 ± 0.015	0.276 ± 0.014	0.277 ± 0.016	0.242 ± 0.008	0.251 ± 0.014
Relative	8.419 ± 0.516	7.363 ± 0.487	7.911 ± 0.447	8.331 ± 0.618	7.387 ± 0.244	7.502 ± 0.336
R. Testis						
Absolute	0.113 ± 0.003	0.121 ± 0.003	0.115 ± 0.006	0.116 ± 0.003	0.110 ± 0.005	0.119 ± 0.002
Relative	3.329 ± 0.088	3.357 ± 0.089	3.282 ± 0.158	3.454 ± 0.075	3.356 ± 0.132	3.571 ± 0.069
Thymus						
Absolute	0.049 ± 0.003	0.051 ± 0.004	0.047 ± 0.003	0.047 ± 0.002	0.044 ± 0.001	0.048 ± 0.002
Relative	1.441 ± 0.086	1.426 ± 0.111	1.361 ± 0.099	1.377 ± 0.044	1.341 ± 0.041	1.440 ± 0.060
Female						
n	9	10	9	10	10	10
Necropsy body wt	31.6 ± 0.8	31.6 ± 1.2	31.5 ± 0.9	31.3 ± 0.8	30.0 ± 1.0	29.0 ± 0.4
Heart						
Absolute	0.153 ± 0.011	0.166 ± 0.010	0.147 ± 0.003	0.185 ± 0.009*	0.155 ± 0.003	0.161 ± 0.003
Relative	4.864 ± 0.341	5.267 ± 0.258	4.691 ± 0.115	5.955 ± 0.362	5.205 ± 0.135	5.542 ± 0.093
R. Kidney						
Absolute	0.216 ± 0.004	0.222 ± 0.005	0.214 ± 0.006	0.211 ± 0.005	0.223 ± 0.004	0.228 ± 0.004
Relative	6.868 ± 0.197	7.051 ± 0.136	6.798 ± 0.139	6.754 ± 0.133	7.477 ± 0.189*	7.862 ± 0.139**
Liver						
Absolute	1.556 ± 0.031	1.593 ± 0.056	1.547 ± 0.049	1.591 ± 0.030	1.525 ± 0.051	1.526 ± 0.038
Relative	49.4 ± 0.9	50.5 ± 0.9	49.1 ± 1.1	51.0 ± 1.2	50.8 ± 0.8	52.6 ± 0.8
Lung						
Absolute	0.326 ± 0.014	0.349 ± 0.018	0.341 ± 0.020	0.303 ± 0.017	0.313 ± 0.014[b]	0.280 ± 0.010
Relative	10.399 ± 0.513	11.092 ± 0.544	10.746 ± 0.380	9.806 ± 0.720	10.427 ± 0.558[b]	9.690 ± 0.379
Thymus						
Absolute	0.060 ± 0.003	0.059 ± 0.005	0.056 ± 0.002	0.052 ± 0.002	0.055 ± 0.003	0.053 ± 0.002
Relative	1.921 ± 0.098	1.849 ± 0.139	1.794 ± 0.077	1.662 ± 0.048	1.816 ± 0.072	1.836 ± 0.092

* Significantly different (P≤0.05) from the vehicle control group by Williams' or Dunnett's test
** P≤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 F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 6-Month Dermal Study of Pentaerythritol Triacrylate[a]

	Vehicle Control	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg
Male						
n	12	14	15	15	12	10
Necropsy body wt	35.5 ± 1.3	31.8 ± 0.7	32.3 ± 1.6	33.8 ± 0.7	31.0 ± 0.4*	33.5 ± 0.7
Heart						
Absolute	0.218 ± 0.011	0.212 ± 0.006	0.218 ± 0.008	0.232 ± 0.006	0.249 ± 0.014	0.264 ± 0.020**
Relative	6.208 ± 0.320	6.694 ± 0.188	6.935 ± 0.367	6.911 ± 0.229	8.032 ± 0.448**	7.887 ± 0.544**
R. Kidney						
Absolute	0.318 ± 0.011	0.302 ± 0.014	0.285 ± 0.012	0.304 ± 0.008	0.307 ± 0.009	0.309 ± 0.008
Relative	9.055 ± 0.361	9.482 ± 0.370	8.926 ± 0.275	9.061 ± 0.287	9.910 ± 0.300	9.277 ± 0.283
Liver						
Absolute	1.788 ± 0.075	1.663 ± 0.048	1.657 ± 0.093	1.811 ± 0.035	1.893 ± 0.036	2.071 ± 0.143*
Relative	51.2 ± 2.7	52.3 ± 1.1	51.0 ± 1.1	53.8 ± 0.9	61.0 ± 1.3**	61.6 ± 3.2**
Lung						
Absolute	0.333 ± 0.024	0.340 ± 0.013	0.308 ± 0.013[b]	0.342 ± 0.013	0.238 ± 0.010**	0.277 ± 0.020**
Relative	9.510 ± 0.673	10.679 ± 0.316	9.847 ± 0.470[b]	10.162 ± 0.363	7.685 ± 0.331*	8.273 ± 0.604*
R. Testis						
Absolute	0.085 ± 0.004	0.088 ± 0.002	0.089 ± 0.003	0.087 ± 0.003	0.077 ± 0.003	0.083 ± 0.004
Relative	2.411 ± 0.146	2.760 ± 0.059	2.834 ± 0.143	2.596 ± 0.103	2.471 ± 0.103	2.490 ± 0.140
Thymus						
Absolute	0.028 ± 0.003	0.031 ± 0.003	0.026 ± 0.003	0.027 ± 0.002	0.018 ± 0.002	0.025 ± 0.003
Relative	0.794 ± 0.076	0.956 ± 0.076	0.782 ± 0.072	0.785 ± 0.050	0.584 ± 0.055	0.750 ± 0.076
Female						
n	12	14	12	12	13	9
Necropsy body wt	29.0 ± 0.9	27.9 ± 0.6	26.4 ± 1.0	25.8 ± 0.7*	26.6 ± 0.8	26.0 ± 1.1
Heart						
Absolute	0.206 ± 0.006	0.187 ± 0.005	0.176 ± 0.008	0.188 ± 0.007	0.204 ± 0.011	0.213 ± 0.013
Relative	7.156 ± 0.233	6.736 ± 0.190	6.709 ± 0.241	7.296 ± 0.230	7.697 ± 0.352	8.324 ± 0.642
R. Kidney						
Absolute	0.212 ± 0.006	0.209 ± 0.004	0.196 ± 0.006	0.204 ± 0.004	0.226 ± 0.008	0.229 ± 0.010
Relative	7.360 ± 0.219	7.507 ± 0.160	7.478 ± 0.197	7.921 ± 0.144	8.517 ± 0.235**	8.854 ± 0.358**
Liver						
Absolute	1.615 ± 0.054	1.496 ± 0.033	1.404 ± 0.070	1.415 ± 0.047	1.624 ± 0.060	1.560 ± 0.098
Relative	55.8 ± 1.0	53.7 ± 1.0	52.9 ± 1.0	54.9 ± 1.2	60.9 ± 1.2*	59.7 ± 2.2*
Lung						
Absolute	0.328 ± 0.009	0.314 ± 0.012	0.305 ± 0.011	0.294 ± 0.014	0.250 ± 0.012**	0.228 ± 0.014**
Relative	11.356 ± 0.250	11.236 ± 0.398	11.641 ± 0.388	11.427 ± 0.570	9.424 ± 0.415**	8.828 ± 0.525**
Thymus						
Absolute	0.032 ± 0.002	0.036 ± 0.002	0.030 ± 0.003	0.029 ± 0.003	0.023 ± 0.002**	0.022 ± 0.003**
Relative	1.091 ± 0.056	1.273 ± 0.051	1.137 ± 0.078	1.109 ± 0.086	0.852 ± 0.057*	0.844 ± 0.096*

* 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=14

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 Pentaerythritol Triacrylate[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	288 ± 11	275 ± 7	266 ± 5*	259 ± 5**
L. Cauda epididymis	0.141 ± 0.003	0.140 ± 0.004	0.135 ± 0.008	0.145 ± 0.004
L. Epididymis	0.401 ± 0.010	0.391 ± 0.007	0.391 ± 0.008	0.394 ± 0.009
L. Testis	1.43 ± 0.03	1.40 ± 0.01	1.45 ± 0.02	1.43 ± 0.02
Spermatid measurements				
Spermatid heads (10^7/g testis)	149 ± 4	148 ± 6	148 ± 4	136 ± 4
Spermatid heads (10^7/testis)	194 ± 6	191 ± 7	197 ± 5	180 ± 4
Spermatid heads (10^7/g cauda)	459 ± 27	420 ± 25	478 ± 69	440 ± 16
Spermatid heads (10^7/cauda)	64.5 ± 3.7	58.8 ± 4.1	61.1 ± 6.7	63.7 ± 3.1
Epididymal spermatozoal measurements				
Motility (%)	74.0 ± 1.0	72.6 ± 0.8	72.8 ± 1.2	75.1 ± 0.8

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test.
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

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

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	10
Necropsy body wt (g)	174 ± 3	171 ± 3	167 ± 4	166 ± 4
Estrous cycle length (days)	5.00 ± 0.00	4.90 ± 0.10	5.20 ± 0.20	5.94 ± 0.58[b]
Estrous stages (% of cycle)				
Diestrus	50.0	46.7	50.0	48.3
Proestrus	19.2	17.5	17.5	14.2
Estrus	17.5	20.0	17.5	14.2
Metestrus	13.3	15.8	14.2	16.7
Uncertain diagnoses	0.0	0.0	0.8	6.7

[a] Necropsy body weights 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 weights) or Dunn's test (estrous cycle lengths). By multivariate analysis of variance, dosed females did 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.

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

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	33.8 ± 0.5	33.7 ± 1.1	32.8 ± 0.4	33.3 ± 0.5
L. Cauda epididymis	0.014 ± 0.001	0.013 ± 0.001	0.013 ± 0.001	0.012 ± 0.001
L. Epididymis	0.040 ± 0.001	0.039 ± 0.001	0.037 ± 0.001	0.038 ± 0.001
L. Testis	0.11 ± 0.00	0.11 ± 0.00	0.10 ± 0.01	0.11 ± 0.01
Spermatid measurements				
Spermatid heads (10^7/g testis)	198 ± 12	230 ± 12	234 ± 13	210 ± 14
Spermatid heads (10^7/testis)	19 ± 1	23 ± 1*	22 ± 1	22 ± 2
Spermatid heads (10^7/g cauda)	725 ± 55	1,140 ± 261	1,034 ± 67*	1,423 ± 270*
Spermatid heads (10^7/cauda)	10.0 ± 0.9	14.1 ± 2.6	13.1 ± 0.9	16.5 ± 3.0
Epididymal spermatozoal measurements				
Motility (%)	73.0 ± 0.5	72.0 ± 0.6	74.5 ± 1.6	74.8 ± 0.5

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's test (spermatid heads/testis) or Shirley's test (spermatid heads/cauda).

[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 (epididymal spermatozoal measurements).

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

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg
n	9	10	10	10
Necropsy body wt (g)	31.6 ± 0.8	31.3 ± 0.8	30.0 ± 1.0	29.0 ± 0.4
Estrous cycle length (days)	4.28 ± 0.15	4.20 ± 0.13	4.25 ± 0.11	4.10 ± 0.10
Estrous stages (% of cycle)				
Diestrus	25.9	30.8	30.0	25.0
Proestrus	23.1	20.8	19.2	20.8
Estrus	26.9	25.8	29.2	30.0
Metestrus	24.1	22.5	21.7	24.2

[a] Necropsy body weights 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 weights) or Dunn's test (estrous cycle lengths). By multivariate analysis of variance, dosed females did not differ significantly 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

Pentaerythritol Triacrylate

Pentaerythritol triacrylate was obtained in two lots. Lot 05318JW, obtained from Aldrich Chemical Company (Milwaukee, WI), was used during the 2-week studies, and lot HCC0340, obtained from Sartomer Company (Exton, PA), was used during the 3- and 6-month 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 pentaerythritol triacrylate studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a clear, viscous liquid, was identified as pentaerythritol triacrylate using infrared spectroscopy and proton and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with the structure of pentaerythritol triacrylate; the infrared spectra were also consistent with a literature spectrum (*Aldrich*, 1985). The NMR spectra indicated a significant number of structurally related impurities in each lot. The infrared and NMR spectra are presented in Figures H1 through H6.

The purity of lot 05318JW was determined by Galbraith Laboratories, Inc. (Knoxville, TN) using elemental analyses and by the analytical chemistry laboratory using gas chromatography, high-performance liquid chromatography (HPLC), and HPLC with mass spectrometry (HPLC/MS). Moisture content was determined by Galbraith Laboratories using Karl Fischer titration. The purity of lot HCC0340 was determined by the analytical chemistry and study laboratories using HPLC. Gas chromatography was performed using system A (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 (lot 05318JW) or 220 nm (lot HCC0340). 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.

For lot 05318JW, elemental analyses for carbon, hydrogen, and oxygen were in agreement with the theoretical values for pentaerythritol triacrylate. Karl Fischer titration indicated approximately 943 ppm water. Gas chromatography indicated one major peak, five impurities with areas greater than 1% relative to the major peak area, and six impurities with relative areas between 0.5% and 1%. HPLC indicated a major peak, 18 impurities with areas greater than 1% relative to the major peak area, and five impurities with relative areas between 0.5% and 1%; the concentration of pentaerythritol triacrylate was determined to be approximately 10%. HPLC/MS indicated that 8 of the 18 impurity peaks with relative areas greater than 1% included structurally related adducts, dimers, and acrylates as well as trimethylolpropane triacrylate and its related esters and adducts. No substantial amount of 4-methoxyphenol, a stabilizer added to pentaerythritol triacrylate, was detected. The overall purity of lot 05318JW was determined to be approximately 10%, which is representative of commercial-grade pentaerythritol triacrylate.

For lot HCC0340, HPLC indicated a major peak, seven impurity peaks with areas greater than 1% of the major peak area, and nine impurity peaks with relative areas between 0.5% and 1%. By comparison of the retention times of these impurity peaks to those in the HPLC/MS analysis of lot 05318JW, the impurities were tentatively identified as structurally related adducts, dimers, and acrylates as well as trimethylolpropane triacrylate and its related esters and adducts. The overall purity of lot HCC0340 was determined to be approximately 45%.

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 by systems similar to systems A and B. 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. 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 system C. 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. No degradation of the acetone was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared twice (2-week studies) or approximately every 4 weeks by mixing pentaerythritol triacrylate and acetone to give the required concentration (Table H2). The dose formulations were stored for up to 35 days for all studies at room temperature in amber glass bottles with Teflon®-lined lids except dose formulations prepared on September 9 and 12, 1996 (3-month studies), which were stored in amber (rat) or clear (mouse) glass vials with Teflon® septa. 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 and a 400 µg/mL formulation were performed by the study laboratory with gas chromatography by systems similar to system A (6.25 and 100 mg/mL) or B (400 µg/mL). 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 pentaerythritol triacrylate were conducted by the study laboratory using gas chromatography by systems similar to systems A and B. During the 2-week studies, the dose formulations were analyzed once; all dose formulations for rats and mice were within 10% of the target concentration with no value greater than 110% of the target concentration (Table H3). Animal room samples of these dose formulations were also analyzed; all animal room samples for rats and three of five for mice were within 10% of the target concentration. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples of these dose formulations were also analyzed (Table H4). Of the dose formulations analyzed, all 15 for rats and 14 of 15 for mice were within 10% of the target concentration, with no value greater than 104% of the target concentration; 10 of 15 animal room samples for rats and nine of 15 for mice were within 10% of the target concentration. During the 6-month study, the dose formulations were analyzed every 8 or 9 weeks (Table H5). Of the dose formulations analyzed, 14 of 15 were within 10% of the target concentration, with no value greater than 103% of the target concentration; all five animal room samples were also within 10% of the target concentration. The single dose formulation in each of the 3- and 6-month studies that was not within 10% of the target concentration was remixed and reanalyzed and was found to be within 10% of the target concentration. 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).

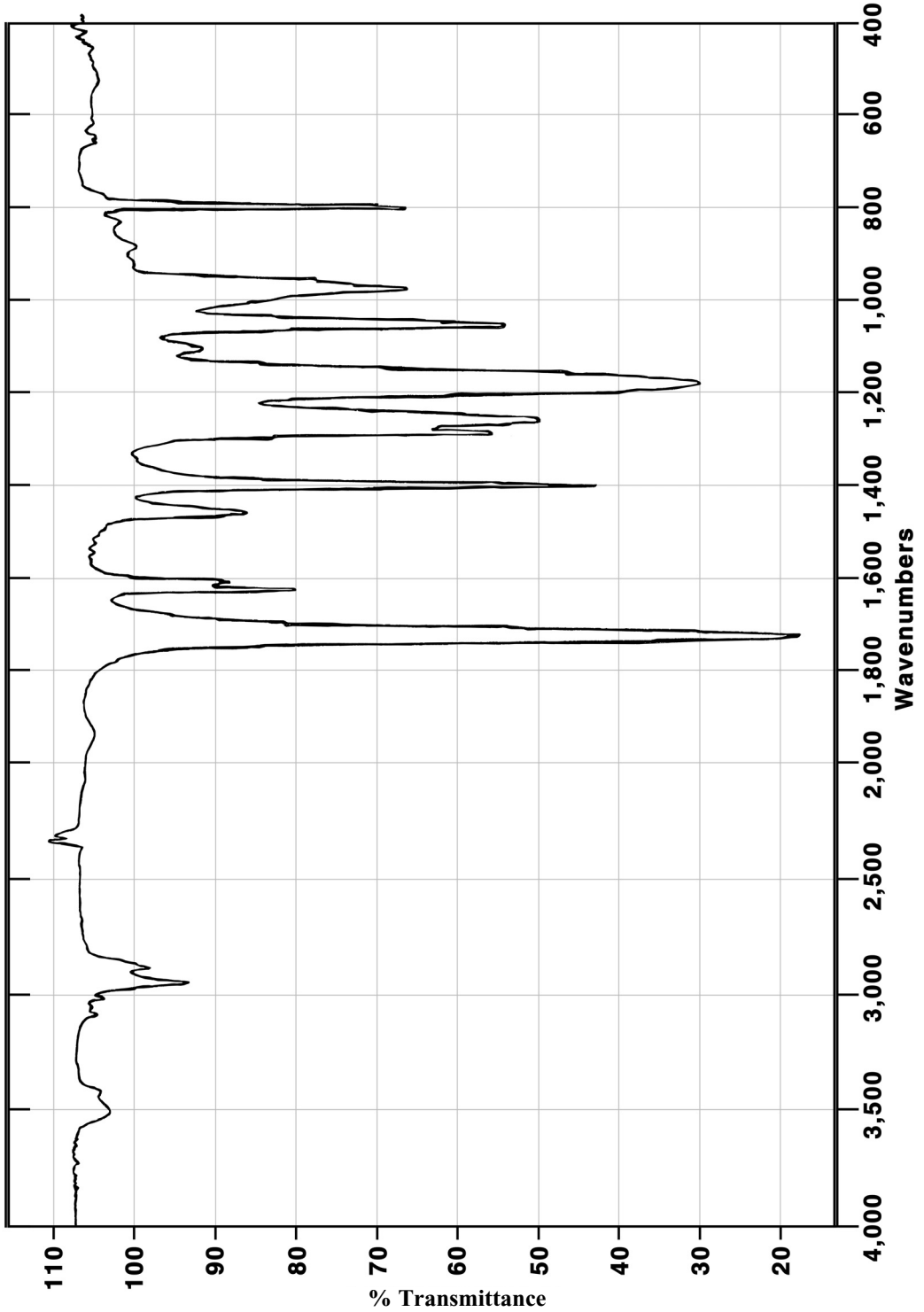

FIGURE H1
Infrared Absorption Spectrum of Pentaerythritol Triacrylate (Approximately 10% Pure Pentaerythritol Triacrylate-Technical Grade) Used in the 2-Week Studies

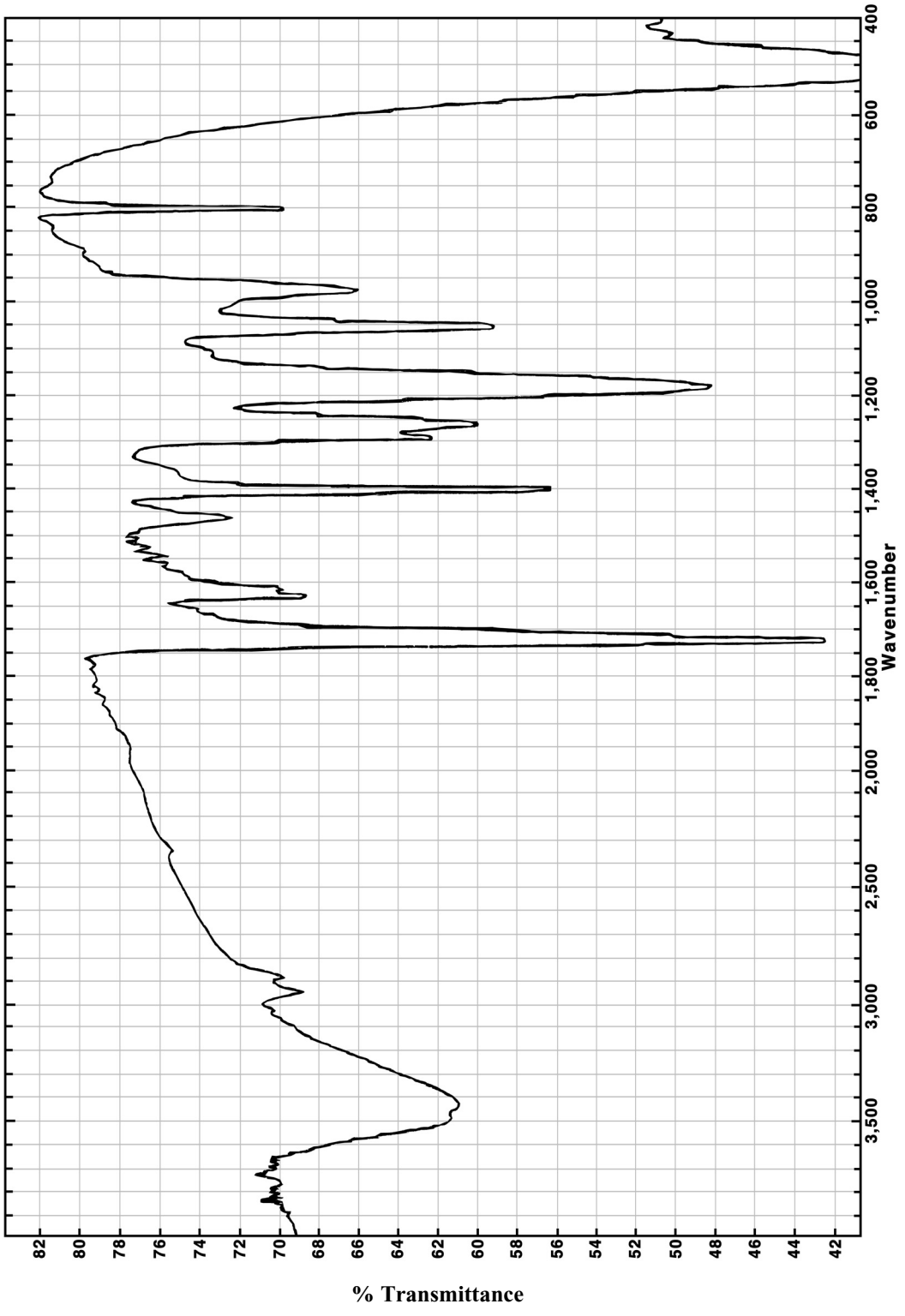

FIGURE H2
Infrared Absorption Spectrum of Pentaerythritol Triacrylate (Approximately 45% Pure Pentaerythritol Triacrylate-Technical Grade) Used in the 3- and 6-Month Studies

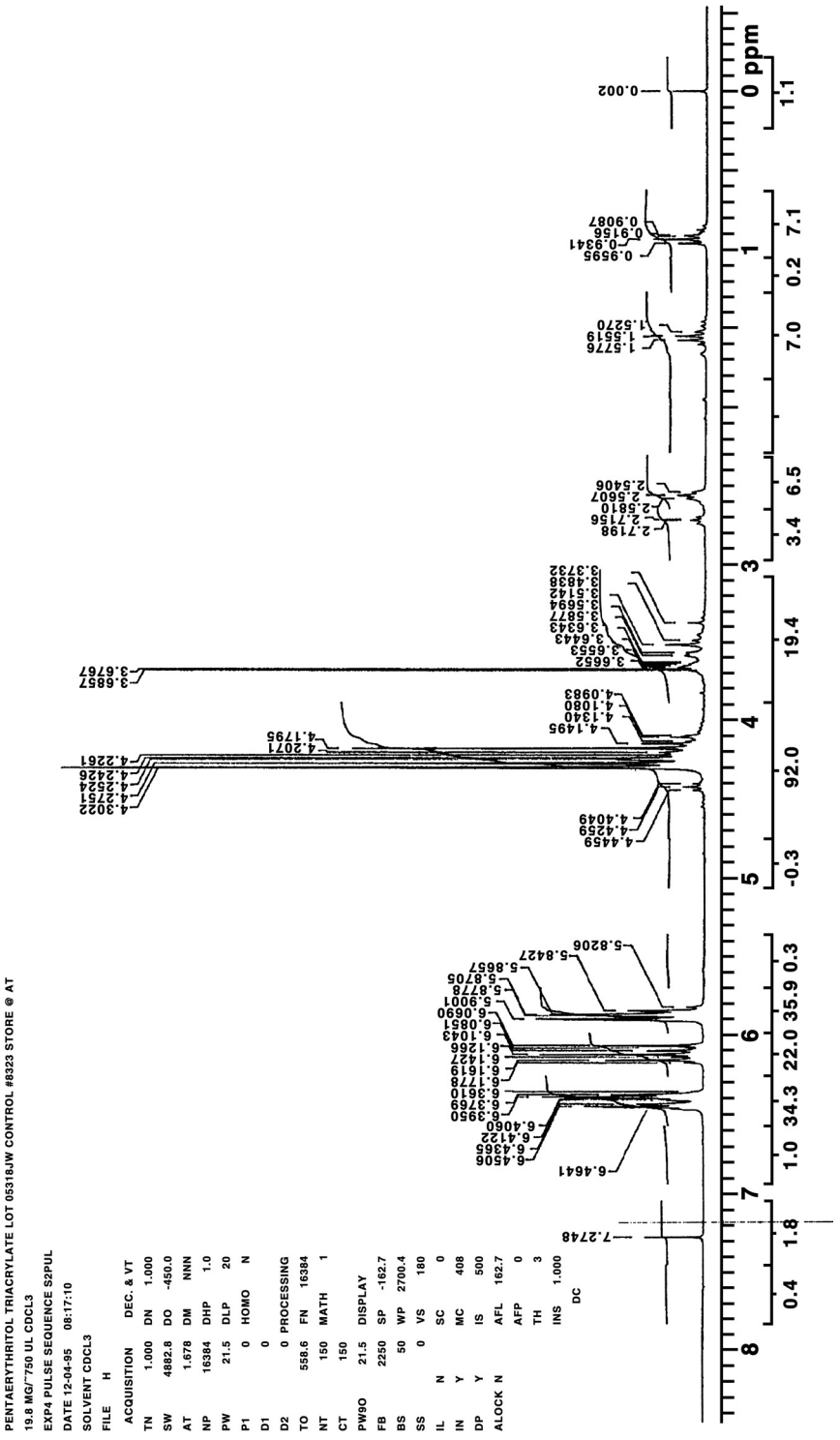

FIGURE H3
Proton Nuclear Magnetic Resonance Spectrum of Pentaerythritol Triacrylate (Approximately 10% Pure Pentaerythritol Triacrylate-Technical Grade) Used in the 2-Week Studies

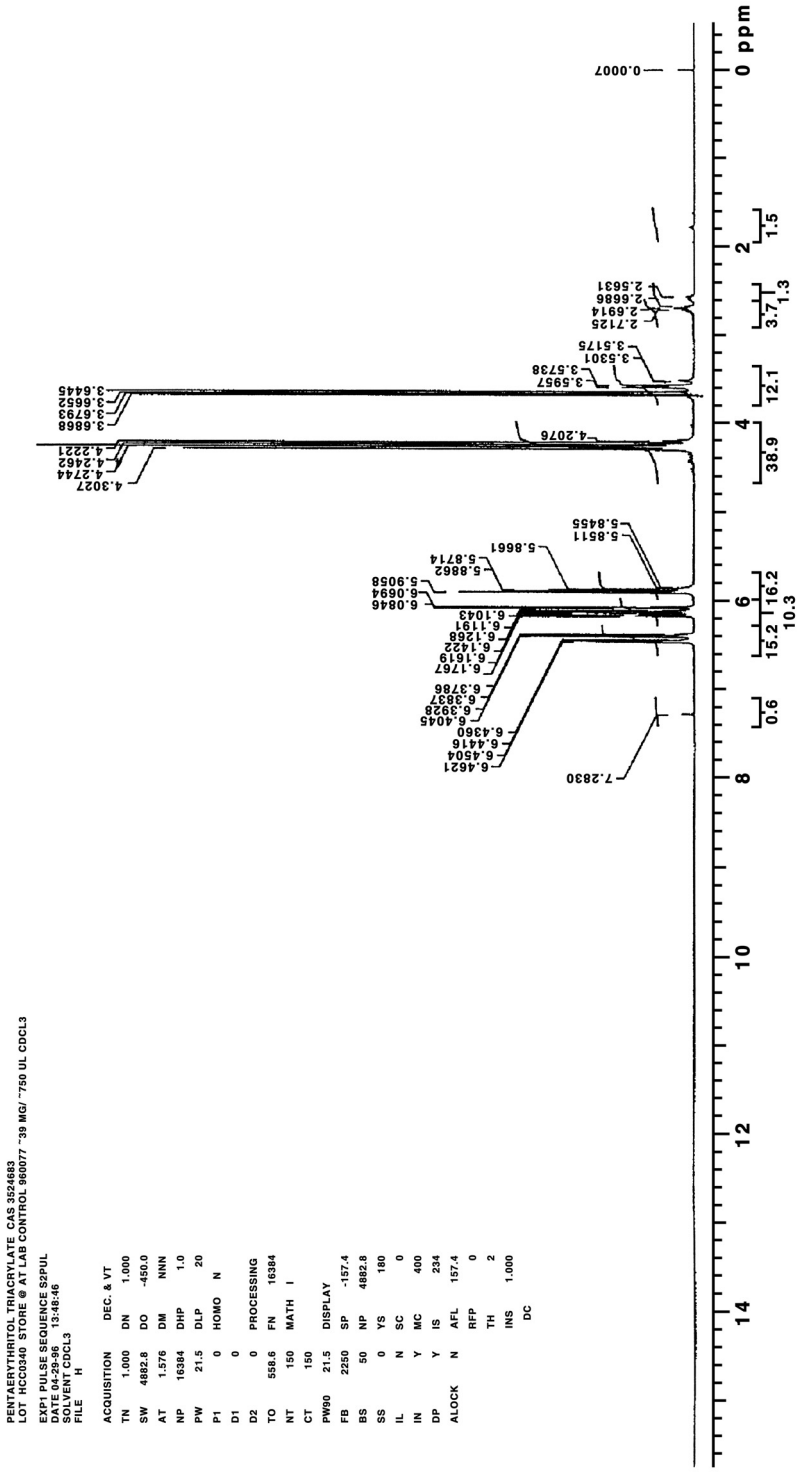

FIGURE H4
Proton Nuclear Magnetic Resonance Spectrum of Pentaerythritol Triacrylate (Approximately 45% Pure Pentaerythritol Triacrylate-Technical Grade) Used in the 3- and 6-Month Studies

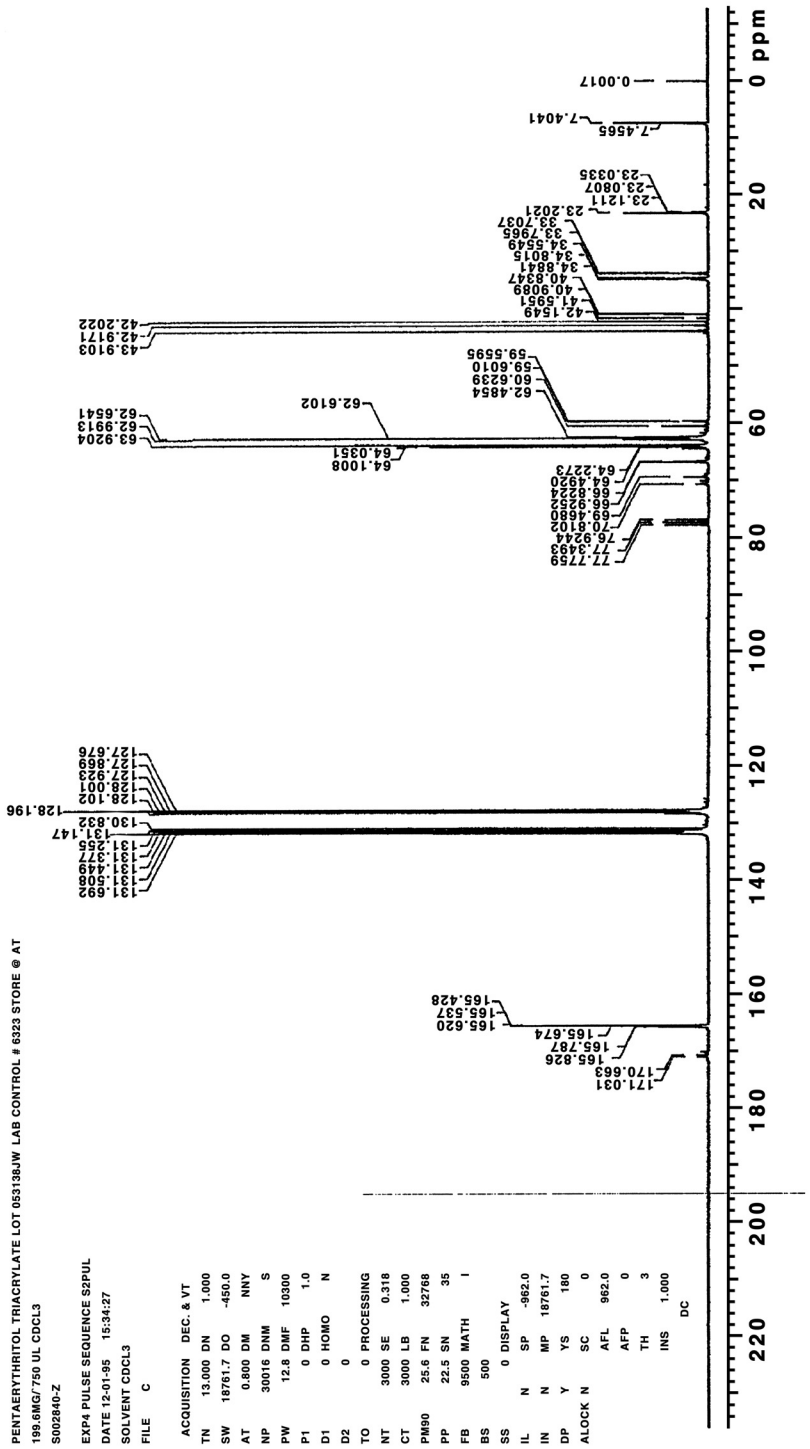

FIGURE H5
^{13}C Nuclear Magnetic Resonance Spectrum of Pentaerythritol Triacrylate (Approximately 10% Pure Pentaerythritol Triacrylate-Technical Grade) Used in the 2-Week Studies

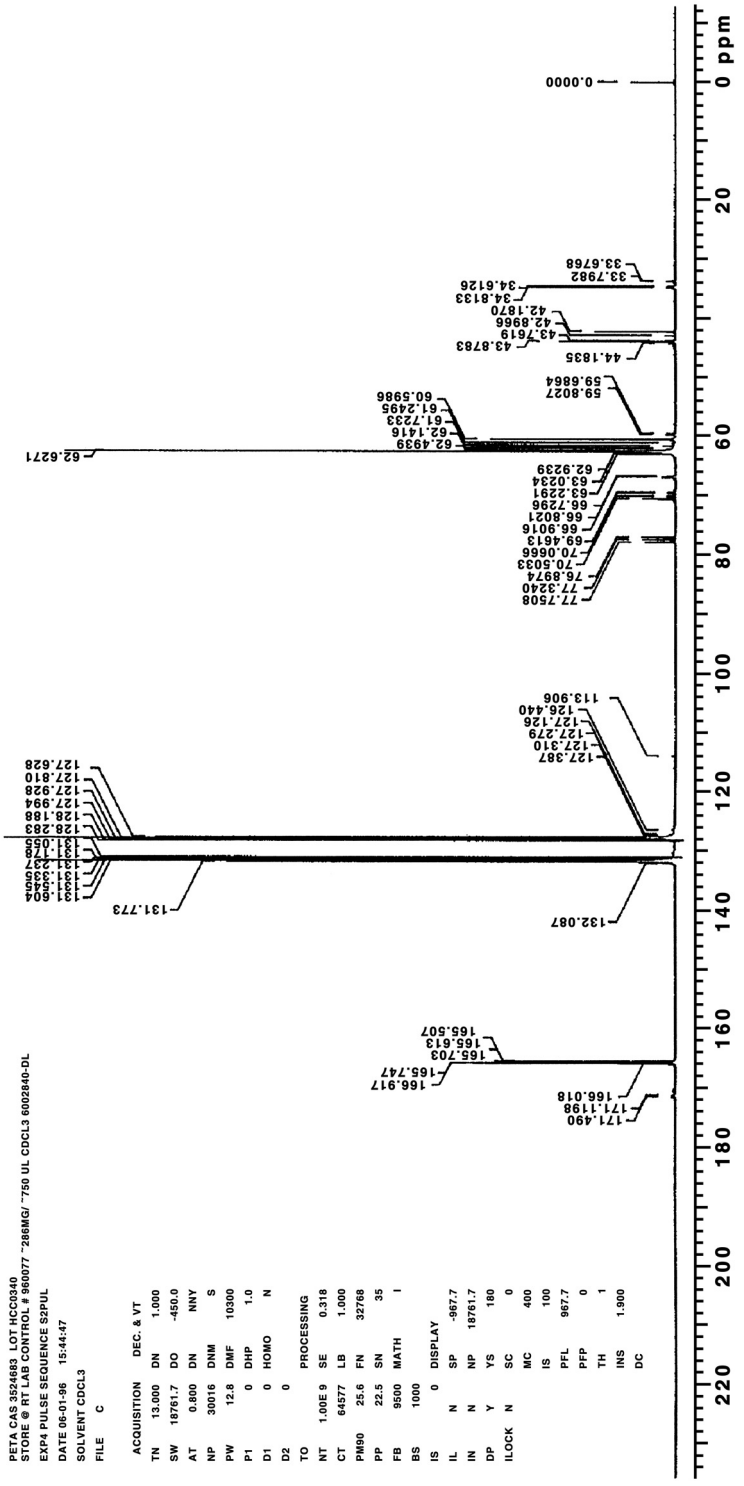

FIGURE H6
^{13}C Nuclear Magnetic Resonance Spectrum of Pentaerythritol Triacrylate (Approximately 45% Pure Pentaerythritol Triacrylate-Technical Grade) Used in the 3- and 6-Month Studies

TABLE H1
Gas Chromatography Systems Used in the Dermal Studies of Pentaerythritol 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 1500 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

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

2-Week Studies	3-Month Studies	6-Month Study
Preparation		
Pentaerythritol 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
Chemical Lot Number		
05318JW	HCC0340	HCC0340
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 9 and 12, 1996, were stored in amber or clear glass vials with Teflon® septa at room temperature; 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 Pentaerythritol Triacrylate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
May 17, 1996	May 18, 1996	25	25.79	+3
		50	52.92	+6
		100	108.7	+9
		200	219.5	+10
		400	431.8	+8
	June 13, 14, and 17, 1996[b]	25	26.61	+6
		50	54.15	+8
		100	103.6	+4
		200	207.0	+4
		400	421.2	+5
Mice				
May 13 and 17, 1996	May 18, 1996	6.25	6.314	+1
		12.5	13.09	+5
		25	25.79	+3
		50	52.92	+6
		100	108.7	+9
	June 13, 14, and 17, 1996[b]	6.25	6.626	+6
		12.5	13.02	+4
		25	25.84[c]	+3
		50	56.65	+13
		100	114.6	+15

[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

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

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
September 9, 1996	September 10-12, 1996	1.5	1.552	+3
		3	3.117	+4
		6	6.269	+4
		12	12.37	+3
		24	24.75	+3
	October 18, 1996[b]	1.5	1.488	–1
		3	3.111	+4
		6	6.351	+6
		12	12.57	+5
		24	25.10	+5
November 4, 1996	November 5, 1996	1.5	1.562	+4
		3	3.078	+3
		6	6.046	+1
		12	11.85	–1
		24	24.15	+1
	December 12-13, 1996[b]	1.5	1.766	+18
		3	5.231	+74
		6	7.219	+20
		12	15.62	+30
		24	28.05	+17
December 2, 1996	December 3, 1996	1.5	1.563	+4
		3	3.105	+4
		6	6.142	+2
		12	12.30	+3
		24	24.46	+2
	January 2, 1997[b]	1.5	1.485	–1
		3	2.942	–2
		6	5.987	0
		12	12.21	+2
		24	23.03	–4

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

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
September 9, 1996	September 10, 1996	0.375	0.3535	–6
		0.75	0.4043[c]	–46
		1.5	1.552	+3
		3	3.117	+4
		6	6.269	+4
September 12, 1996	September 12, 1996	0.75	0.7473[d]	0
	October 18, 1996[b]	0.375	0.3333	–11
		0.75	0.7502	0
		1.5	1.539	+3
		3	3.153	+5
		6	6.528	+9
November 4, 1996	November 5, 1996	0.375	0.3883	+4
		0.75	0.7628	+2
		1.5	1.562	+4
		3	3.078	+3
		6	6.046	+1
	December 12-13, 1996[b]	0.375	0.4420	+18
		0.75	0.9022	+20
		1.5	1.857	+24
		3	3.620	+21
		6	8.960	+49
December 2, 1996	December 3, 1996	0.375	0.3842	+2
		0.75	0.7695	+3
		1.5	1.563	+4
		3	3.105	+4
		6	6.142	+2
	January 2, 1997[b]	0.375	0.3456	–8
		0.75	0.7414	–1
		1.5	1.504	0
		3	2.914	–3
		6	6.004	0

[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] Animal room samples
[c] Remixed; not used in study
[d] Results of remix

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

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
July 13, 1998	July 13-14, 1998	0.227	0.2288	+1
		0.455	0.4606	+1
		0.909	0.9139	+1
		1.82	1.772	–3
		3.64	3.623	0
	August 24-25, 1998[b]	0.227	0.2339	+3
		0.455	0.4511	–1
		0.909	0.9422	+4
		1.82	1.979	+9
		3.64	3.680	+1
September 8, 1998	September 8-10, 1998	0.227	0.1904[c]	–16
		0.455	0.4149	–9
		0.909	0.8249	–9
		1.82	1.722	–5
		3.64	3.575	–2
September 13, 1998	September 14, 1998	0.227	0.2224[d]	–2
November 30, 1998	December 1-2, 1998	0.227	0.2220	–2
		0.455	0.4464	–2
		0.909	0.9247	+2
		1.82	1.880	+3
		3.64	3.694	+1

[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] Animal room samples
[c] Remixed; not used in study
[d] Results of remix

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., or BioReliance Corporation (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
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
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
M. arthritidis	Study termination
Parvovirus	4 weeks, study termination

RESULTS

One male and two female rats in the 3-month studies had a positive titer for *H. hepaticus*. Also, one male and one female rat in the 3-month studies were equivocal for *H. hepaticus*. For the 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 pentaerythritol triacrylate to induce contact hypersensitization. Primary irritancy studies of approximately 10% and 45% pentaerythritol triacrylate mixtures were performed to screen for toxicity and determine the maximal nonirritating and minimal irritating concentrations of pentaerythritol triacrylate. Mouse ear swelling tests and local lymph node assays of the two mixtures were then conducted to assess 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

Pentaerythritol triacrylate mixtures were obtained from Aldrich Chemical Company (Milwaukee, WI; lot 05318JW, approximately 10% pentaerythritol triacrylate) and Sartomer Company (Exton, PA; lot HCC0340, approximately 45% pentaerythritol triacrylate). Acetone was used as the vehicle. Analyses of the bulk chemicals are described in Appendix H. Dose formulations were prepared daily by mixing pentaerythritol triacrylate in acetone.

Female BALB/c mice were obtained from the National Cancer Institute (Bethesda, MD) or Charles River Laboratories (Wilmington, MA). The mice were approximately 6 to 9 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 pulmonis*) 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 studies (Figure K1), groups of four mice received 50 µL dermal applications of 0% (vehicle controls), 0.1%, 0.25%, 0.5%, 1%, 3%, or 5% (w/v) of the approximately 10% pentaerythritol triacrylate mixture or 0%, 0.025%, 0.05%, 0.075%, 0.1%, 0.25%, or 0.5% of the approximately 45% pentaerythritol triacrylate mixture 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. For each study, 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 tests (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) of each pentaerythritol triacrylate mixture 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% of the appropriate pentaerythritol triacrylate mixture (background controls). The three groups sensitized with pentaerythritol triacrylate received a challenge dose of 0.25% of the appropriate pentaerythritol triacrylate mixture. 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 assays (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) of each pentaerythritol triacrylate mixture in acetone. When a no-effect level was not determined in the first study of the approximately 10% mixture, that study was repeated

using doses of 0.005%, 0.01%, and 0.05% of the approximately 10% pentaerythritol mixture 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 approximately 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 pentaerythritol triacrylate treatment in dosed mice. Results of the irritancy studies of the approximately 10% and 45% pentaerythritol triacrylate mixtures indicated that the maximal nonirritating and minimal irritating doses were 0.1% and 0.25% pentaerythritol triacrylate, respectively, for each mixture (Figures K4 and K5).

The mouse ear swelling test did not indicate the approximately 10% pentaerythritol triacrylate mixture as a contact sensitizer in female BALB/c mice at the doses tested 24 or 48 hours after dosing (Figure K6). Testing of the approximately 45% pentaerythritol triacrylate mixture with the ear swelling test paradigm indicated pentaerythritol triacrylate as a contact sensitizer at 0.1%, with significant increases (compared to background controls) in percent ear swelling noted 24 and 48 hours postchallenge (Figure K7). In the first local lymph node assay using the approximately 10% pentaerythritol triacrylate mixture, a significant increase in lymph node cell proliferation occurred in all dosed groups of mice compared to the vehicle controls; in the second assay using lower sensitizing doses, a significant response occurred only in the 0.05% group (Figure K8). The local lymph node assay of the approximately 45% pentaerythritol triacrylate mixture yielded a positive response only with a sensitizing concentration of 0.25% (Figure K9).

In summary, for both the approximately 10% and 45% pentaerythritol triacrylate mixtures, the maximal nonirritating concentration was determined to be 0.1%, and the minimal irritating concentration to be 0.25%. The mouse ear swelling test yielded negative results for pentaerythritol triacrylate as a potential contact sensitizer when the approximately 10% pentaerythritol triacrylate mixture was used and positive results when the approximately 45% pentaerythritol triacrylate mixture was used. Positive responses were seen in local lymph node assays at concentrations of 0.05%, 0.1%, and 0.25% pentaerythritol triacrylate when the approximately 10% pentaerythritol triacrylate mixture was used and at a concentration of 0.25% pentaerythritol triacrylate when the approximately 45% pentaerythritol triacrylate mixture was used. These data indicate the potential of pentaerythritol triacrylate as a weak contact sensitizer.

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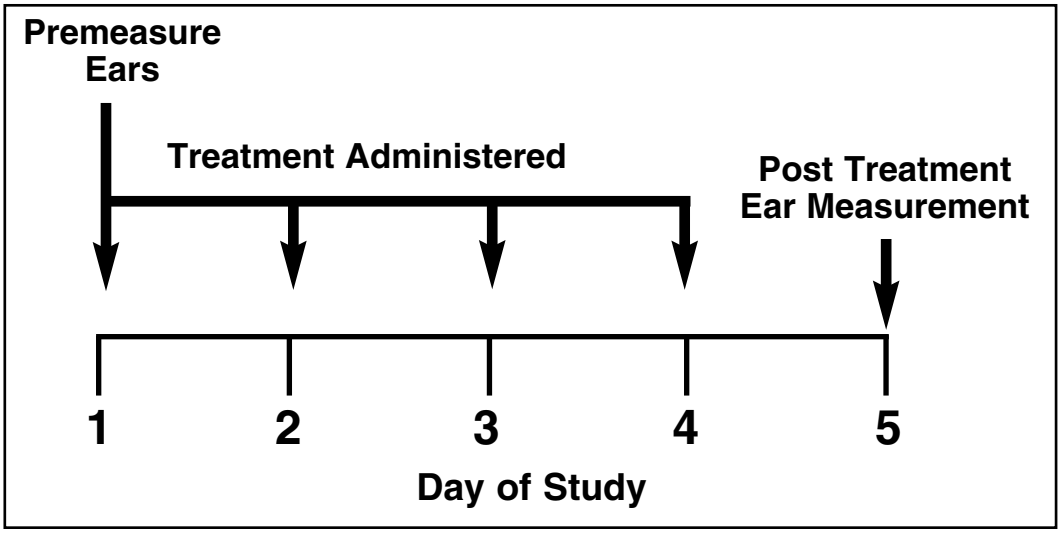

FIGURE K1
Major Events in the Primary Irritancy Studies

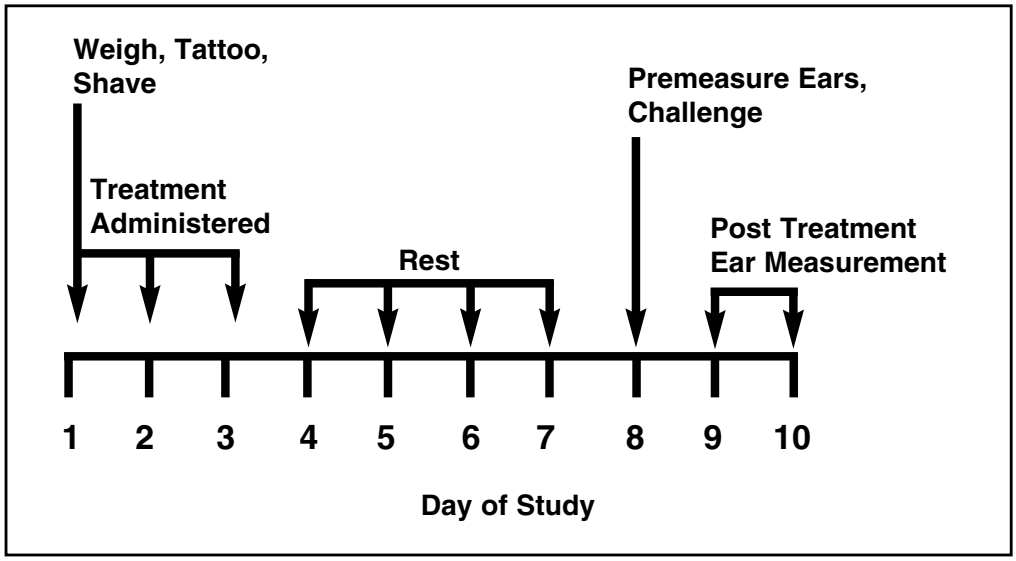

FIGURE K2
Major Events in the Mouse Ear Swelling Tests

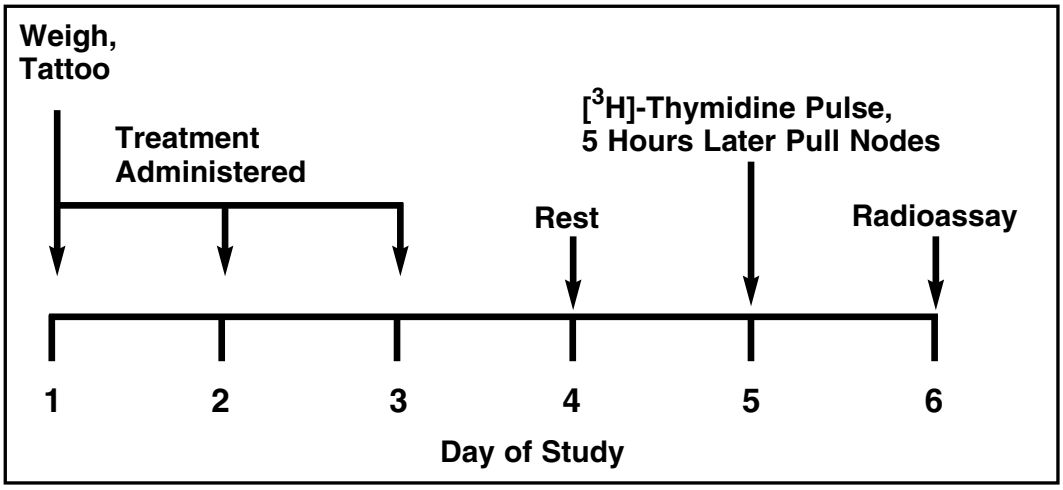

FIGURE K3
Major Events in the Local Lymph Node Assays

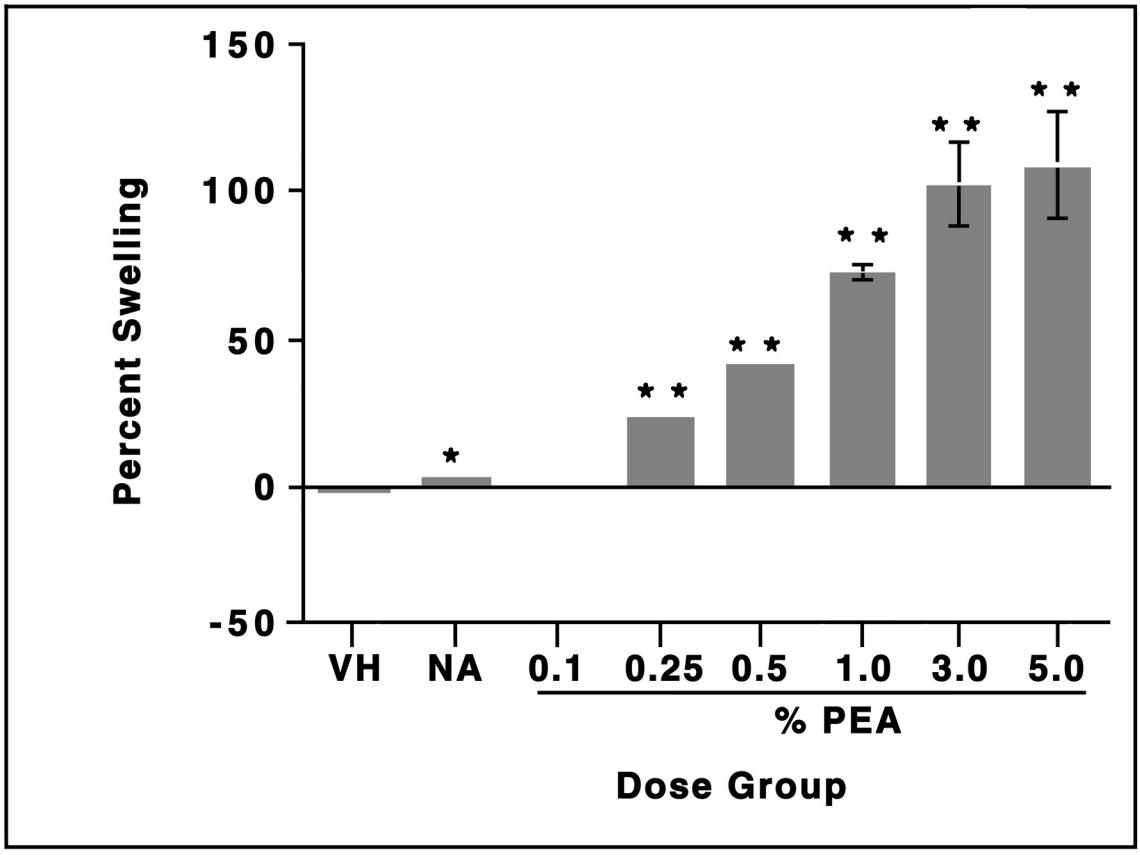

FIGURE K4
Primary Irritancy Response to the Approximately 10% Pentaerythritol Triacrylate Mixture in Female BALB/c Mice
* Statistically significant ($P<0.05$) compared to vehicle control
**$P<0.01$
Means for each group are shown, with bars representing the standard error.
VH=vehicle controls, NA=untreated (naive) controls,
PEA=approximately 10% pentaerythritol triacrylate mixture

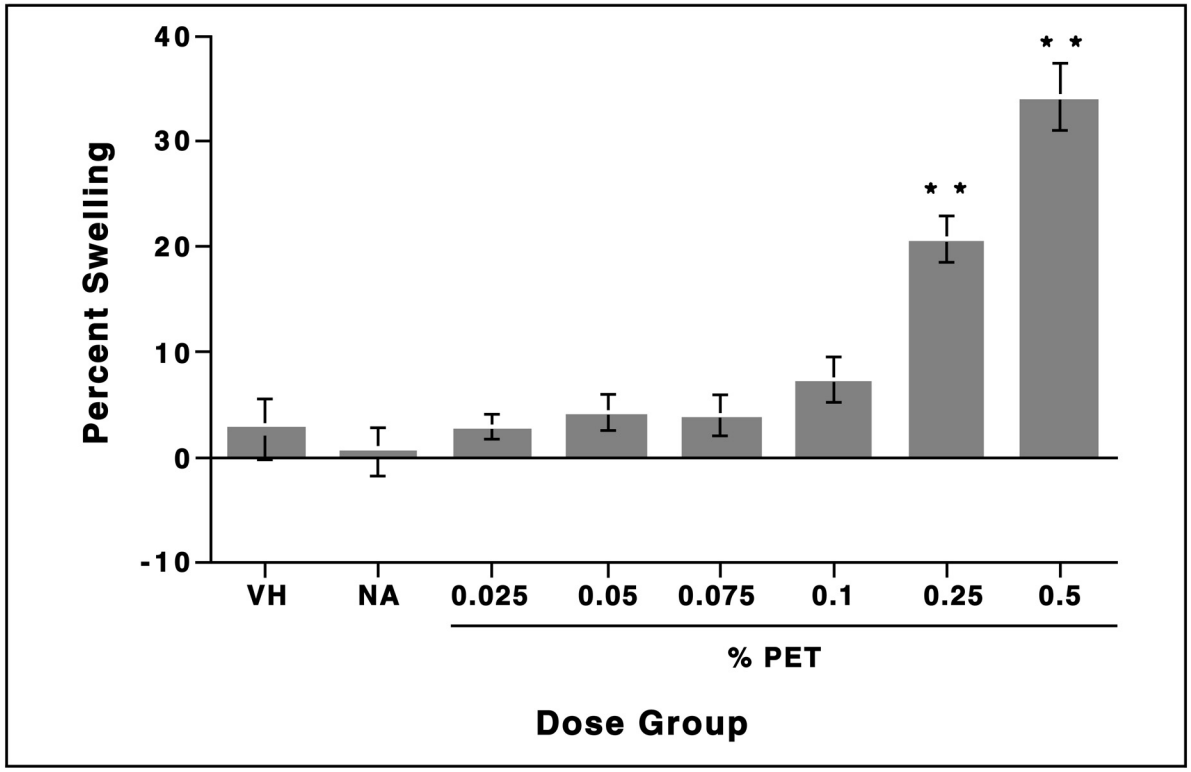

Figure K5
Primary Irritancy Response to the Approximately 45% Pentaerythritol Triacrylate Mixture in Female BALB/c Mice
**Statistically significant (P<0.01) compared to vehicle control
Means for each group are shown, with bars representing the standard error.
VH=vehicle controls, NA=untreated (naive) controls,
PET=approximately 45% pentaerythritol triacrylate mixture

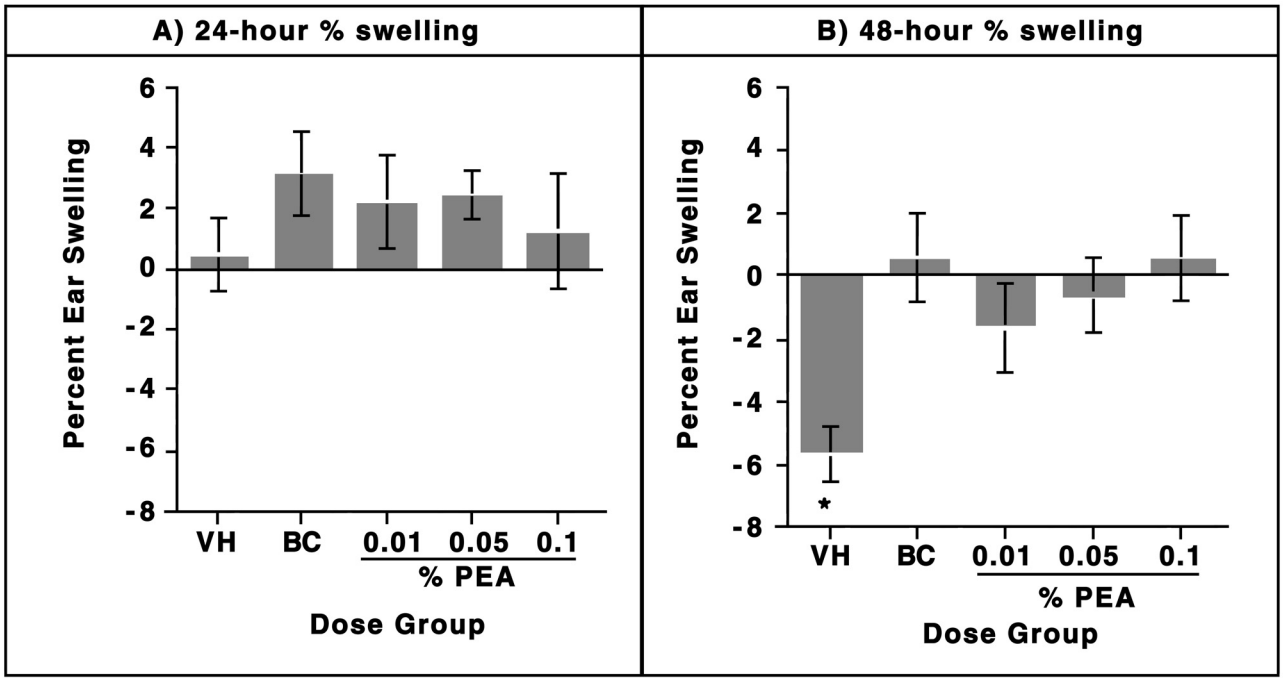

FIGURE K6
Contact Hypersensitivity Response to the Approximately 10% Pentaerythritol Triacrylate Mixture in Female BALB/c Mice (Mouse Ear Swelling Test)
Means for each group are shown, with bars representing the standard error.
VH=vehicle controls, BC=background controls,
PEA=approximately 10% pentaerythritol triacrylate mixture

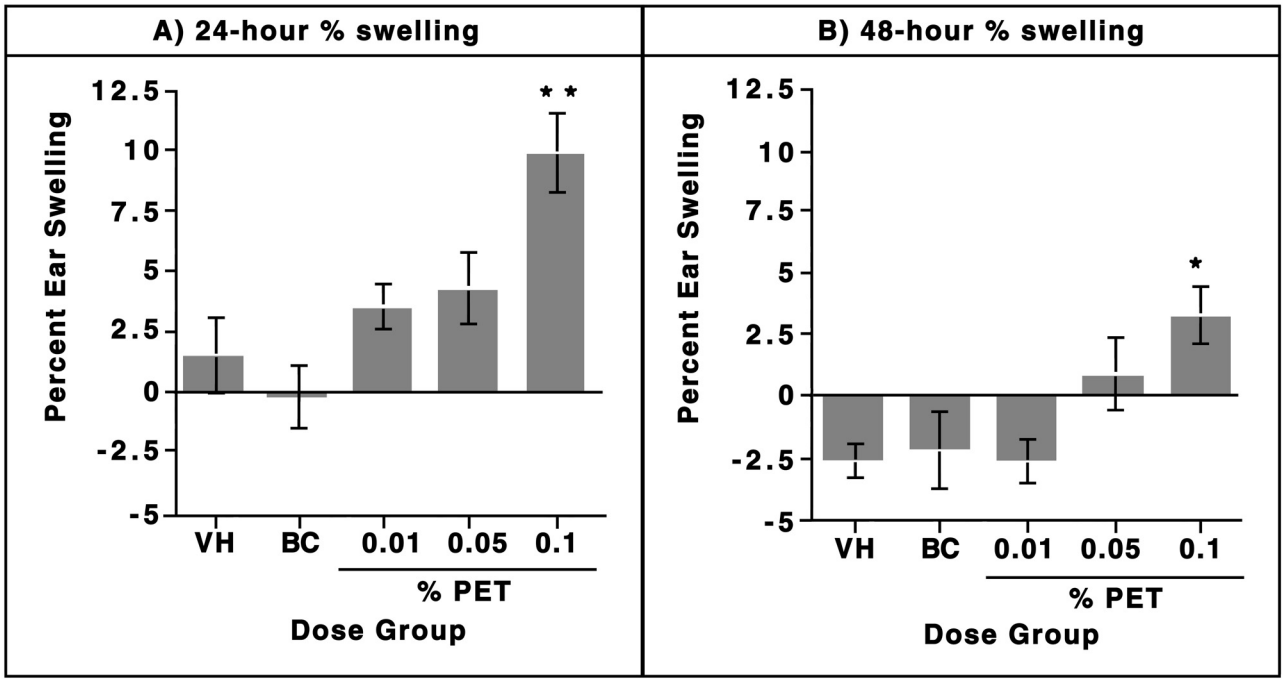

Figure K7
Contact Hypersensitivity Response to the Approximately 45% Pentaerythritol Triacrylate Mixture in Female BALB/c Mice (Mouse Ear Swelling Test)
* Statistically significant ($P<0.05$) compared to background control
** $P<0.01$
Means for each group are shown, with bars representing the standard error.
VH=vehicle controls, BC=background controls,
PET=approximately 45% pentaerythritol triacrylate mixture

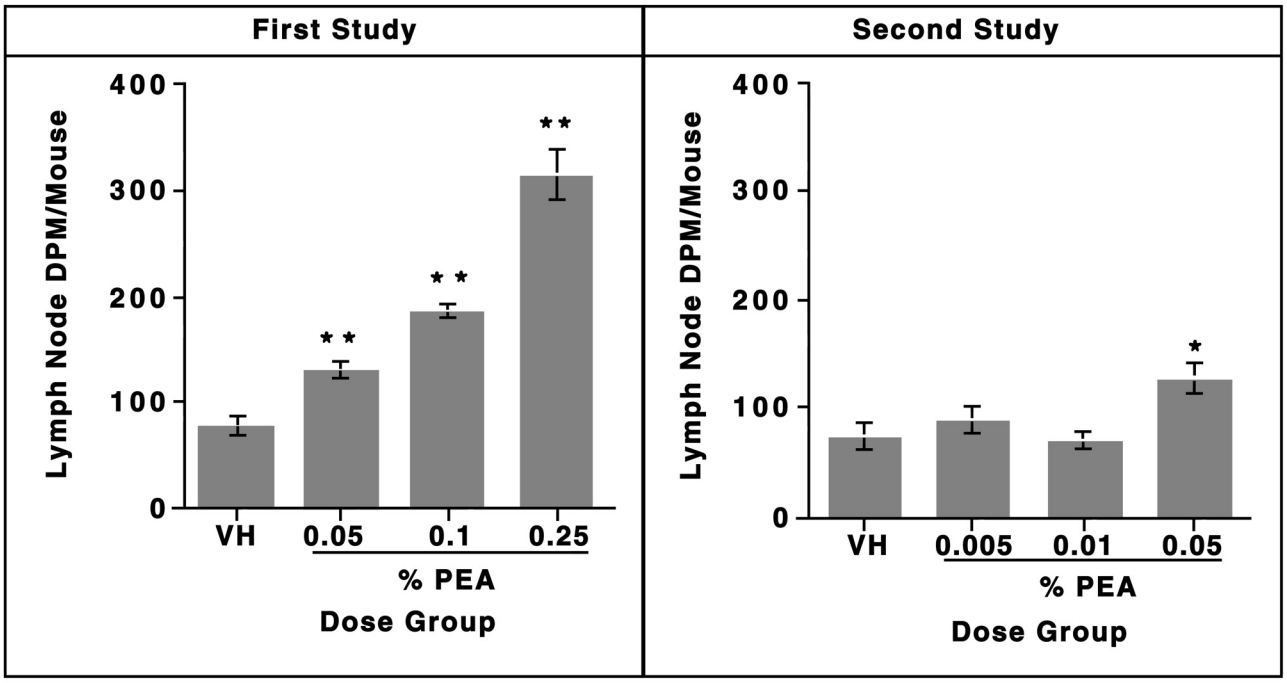

FIGURE K8
Contact Hypersensitivity Response to the Approximately 10% Pentaerythritol Triacrylate Mixture in Female BALB/c Mice (Local Lymph Node Assays)
* Statistically significant ($P<0.05$) compared to background control
** $P<0.01$
Means for each group are shown, with bars representing the standard error.
VH=vehicle controls, BC=background controls,
PEA=approximately 10% pentaerythritol triacrylate mixture

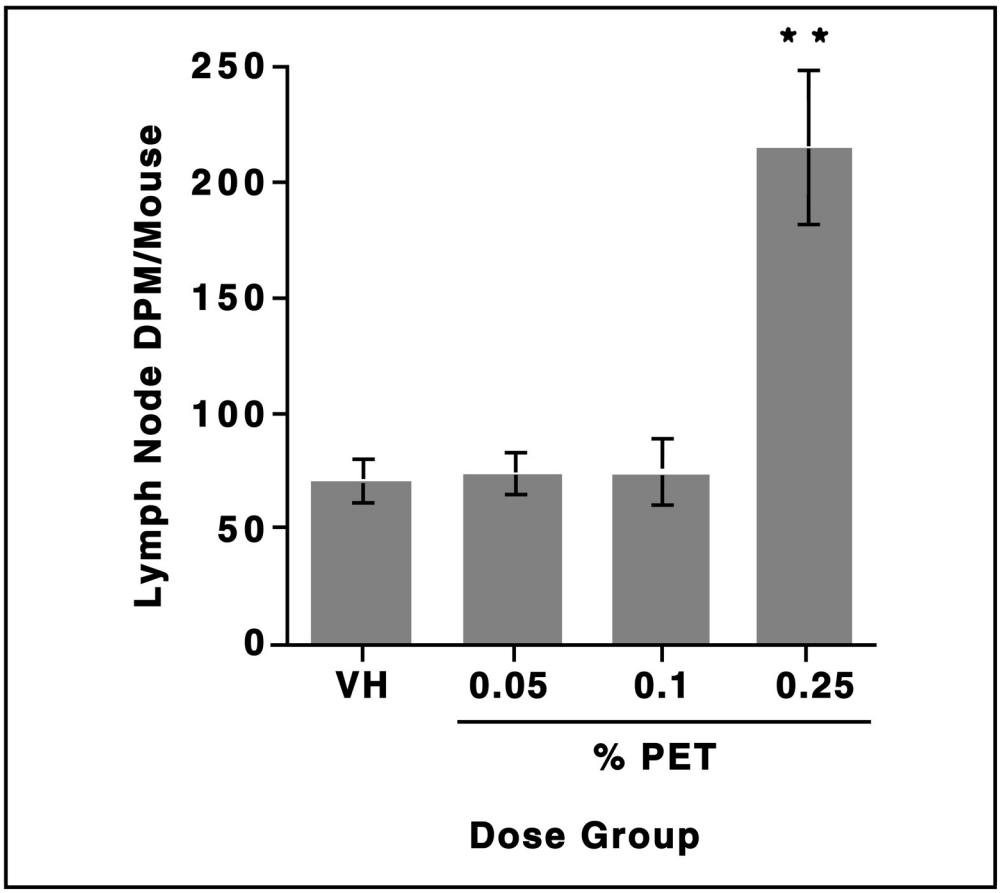

FIGURE K9
Contact Hypersensitivity Response to the Approximately 45% Pentaerythritol Triacrylate Mixture in Female BALB/c Mice (Local Lymph Node Assay)
** Statistically significant (P<0.01) compared to background control
Means for each group are shown, with bars representing the standard error.
VH=vehicle controls, PET=approximately 45% pentaerythritol triacrylate mixture