National Toxicology Program
Toxicity Report Series
Number 15

NTP Technical Report On Toxicity Studies of

t-Butyl Perbenzoate

(CAS NUMBER: 614-45-9)

Administered by Gavage to F344/N Rats and B6C3F$_1$ Mice

H.B. Matthews, PhD, Study Scientist
National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

NIH Publication No. 92-3134
July 1992

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

NOTE TO THE READER

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. In July, 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from the 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 toxicity study report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical 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.

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CONTRIBUTORS

The NTP report on the toxicity studies of t-butyl perbenzoate is based primarily on 14-day and 13-week studies performed over the period between March, 1985, and July, 1988, at Battelle Memorial Laboratories, Columbus, OH; and on disposition and stability studies performed at Research Triangle Institute, Research Triangle Park, NC.

National Toxicology Program
Evaluated experiment, interpreted results, and reported findings

H.B. Matthews, PhD
Study Scientist
John Bucher, PhD
Leo T. Burka, PhD
Michael Elwell, DVM, PhD
Joel Mahler, DVM
Morrow Thompson, DVM, PhD
Errol Zeiger, PhD

Coordinated Report Preparation

Jane Lambert, BS
Diane Overstreet, BS
Kristine Witt, MS
Oak Ridge Associated Universities

NTP Pathology Working Group
Evaluated slides and prepared pathology report

Katharina Heider, DVM
Chairperson
CIBA-GEIGY
Michael Elwell, DVM, PhD
National Toxicology Program
Jeffrey Everitt, DVM
CIIT
Joel Leininger, DVM, PhD
National Toxicology Program
Margarita McDonald, DVM, PhD
National Toxicology Program
John Peckham, DVM
Experimental Pathology Laboratories, Inc.

Battelle Columbus Laboratories
Principal contributors

Arthur C. Peters, DVM
Principal Investigator
M. Hejtmancik, PhD
Study Director, Toxicologist
Ming Chang, PhD
Chemistry
P. Jepsen, DVM, MS
Study Veterinarian
L. Mezza, DVM, MS
Pathology

Research Triangle Institute
Conducted disposition studies

Robert Jeffcoat, PhD
Principal Investigator
Monroe Wall, PhD
Co-Study Director
Thorir Bjornsson, PhD
Pharmacokinetics
Donald Feldman, DVM
Study Veterinarian
Timothy Tippin, BA
Chemist

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

John Peckham, DVM

Analytical Sciences, Inc.
Provided statistical analysis

Richard Morris, MS
Steven Seilkop, MS
Janet Teague, MS

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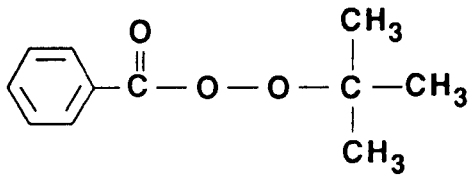
t-BUTYL PERBENZOATE



Molecular Formula: $C_{11}H_{14}O_3$

CAS Number: 614-45-9

Molecular Weight: 194.25

Synonyms: Benzenecarboperoxoic acid; 1,1-dimethylester; Esperox 10; Trigonox C; t-BP.

ABSTRACT

t-Butyl perbenzoate (t-BP) is a relatively stable, lipid-soluble, organic peroxide widely used in the polymer industry. Studies were designed to determine the stability of t-BP in various biological media, its dermal absorption and distribution in intact animals, and the toxicity of t-BP when administered orally to both sexes of rats and mice for 14 days or 13 weeks. In genetic toxicity studies, t-BP was found to be mutagenic in *Salmonella typhimurium* strains TA100, TA1537, and TA98, with and without metabolic activation. t-BP-induced sister-chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells *in vitro* but did not induce formation of micronuclei in peripheral blood in mice in the 13-week studies.

Stability studies indicated t-BP was sufficiently stable in dose formulations to permit administration by gavage, intravenous injection, or dermally. However, t-BP degraded rapidly in blood, stomach contents, and liver homogenates, or in the presence of glutathione. Initial degradation products of t-BP are benzoic acid and t-butanol. Studies of t-BP disposition determined that approximately 16% of dermal doses administered to rats was absorbed and rapidly eliminated without tissue accumulation. Similarly, t-BP given intravenously was rapidly degraded and eliminated, primarily in urine, with no apparent accumulation in any tissue. Because dermal absorption was considered insufficient to administer a toxic dose, studies of t-BP toxicity were performed using gavage administration.

Results of 14-day toxicity studies with 5 animals of each sex of rats and mice indicated that t-BP, adminstered by gavage in corn oil in doses ranging from 70 to 1112 mg/kg, produced no marked signs of systemic toxicity. Toxicity in mice, attributable to t-BP, was limited largely to increased stomach weights in males and females receiving the highest doses. This toxicity was characterized by forestomach epithelial hyperplasia, ulceration, and acute inflammation. Equimolar doses of the degradation products of t-BP (t-butanol and benzoic acid) also were administered in the 14-day

studies to determine if t-BP toxicity could be attributed to the parent compound or products of its chemical degradation and/or metabolism. Results of these studies indicated that equimolar doses of t-butanol were not toxic in either sex or species. Some systemic toxicity of benzoic acid was observed in both sexes of mice, but not rats, receiving the highest dose (642 mg/kg). Toxicity was evidenced by the poor condition of dosed animals and in several deaths during the first week of the study. No lesions were observed microscopically, and it is speculated that this toxicity may have been due to acidosis.

In the 13-week studies, t-BP was administered by gavage in water to 10 rats and 10 mice of each sex, at doses up to 500 mg/kg. The doses resulted in depressed body-weight gains in the highest dose groups and in dose-dependent increases in forestomach weights. Hyperplasia of the forestomach mucosa was observed in most groups of dosed rats and increased in severity with dose. Hyperplasia was characterized by increased cellularity and basophilia of the squamous epithelium with variable degrees of hyperkeratosis. t-BP toxicity observed in mice was limited to increased forestomach weight in most dose groups and to less dramatic increases in glandular stomach weight in mice receiving the highest doses. Forestomach toxicity was characterized by dose-dependent increases in hyperplasia of the squamous epithelium in all mice except those in the low dose group.

Based on the results presented in this report, it is concluded that the no-observed-adverse-effect-level (NOAEL) for t-BP to induce forestomach lesions in rats and mice is approximately 30 mg/kg. Systemic toxicity was not observed in either species with oral doses as high as 1112 mg/kg.

PEER REVIEW

Peer Review Panel

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies on t-butyl perbenzoate on March 11-12, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

National Toxicology Program's Board of Scientific Counselors Technical Reports Review Subcommittee

Paul T. Bailey, PhD
Mobil Oil Corporation
Toxicology Division
Princeton, NJ

Daniel S. Longnecker, MD, Chair
Department of Pathology
Dartmouth Medical School
Hanover, NH

Jay I. Goodman, PhD
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, MI

Ellen K. Silbergeld, PhD
University of Maryland Medical School
Baltimore, MD

Ad Hoc Subcommittee Panel of Experts

Louis S. Beliczky, MS, MPH
Department of Industrial Hygiene
United Rubber Workers Intl. Union
87 South High Street
Akron, OH

Gary P. Carlson, PhD
Department of Pharmacology and Toxicology
Purdue University
West Lafayette, IN

Harold Davis, DVM, PhD
School of Aerospace Medicine
Brooks Air Force Base, TX

Robert H. Garman, DVM
Consultants in Veterinary Pathology
Murrysville, PA

David W. Hayden, DVM, PhD
Department of Veterinary Pathobiology
College of Veterinary Medicine
University of Minnesota
St. Paul, MN

Curtis D. Klaassen, PhD
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Barbara McKnight, PhD
Department of Biostatistics
University of Washington
Seattle, WA

Lauren Zeise, PhD
California Department of Health Services
Berkeley, CA

Summary of Peer Review Comments

Dr. H.B. Matthews, NIEHS, NTP Staff Scientist, introduced the short-term toxicity studies of t-butyl perbenzoate (t-BP) by reviewing the uses of t-BP and the rationale for the study, findings from chemical disposition studies, experimental design, and results.

Dr. Hayden, a principal reviewer, said this was a well-documented and clearly written report indicating that t-BP had little or no toxicity to rodents other than changes in the stomach. He cautioned that on a long-term study there might be problems with stomach ulcers or perforating ulcers leading to excessive mortality.

Dr. Bailey, a second principal reviewer, also thought this was a well-performed study and a well-written report. He commented on an apparent contradiction in the draft report, that while the text states that t-BP is not "normally inhaled," it also cites reports of workers exposed to the chemical by inhaling t-BP vapors.

Dr. Davis asked whether the forestomach lesions were focused primarily around the limiting ridge as in some recent studies. Dr. M. Elwell, NIEHS, said that at the higher doses lesions were seen over large areas of the forestomach.

Dr. Carlson noted the disposition studies indicated significant amounts of radiolabel were found in skin taken from sites other than that used for dermal administration. Dr. Matthews indicated that this was a frequent finding in dermal disposition studies performed with volatile chemicals, and that it was not an artifact.

Following a short discussion of editorial and other comments, Dr. Longnecker indicated that the panel would accept the report, with the indicated changes.

INTRODUCTION

t-Butyl perbenzoate (t-BP) is a relatively stable organic peroxide used almost exclusively as a free radical-initiator in the polymer industry. It is one of the catalysts most commonly used to promote polymerization of unsaturated resins such as styrene and vinyl chloride. It also is used in curing unsaturated polyester resins, in polymerizing various monomers, and in crosslinking of polymers. Annual U.S. production is estimated to be 3 to 4 million pounds; another 1 to 2 million pounds are imported (USITC, 1988). An estimated 7,000 to 8,000 workers are exposed to t-BP in the work place; depending upon the job involved, worker exposure may be dermal, or, less commonly, by inhalation or ingestion (Free-radical initiators, 1983-84; Plunkett, 1976).

Like most peroxides, t-BP is very reactive; risks associated with its use are primarily those related to explosion and fire. Direct contact with concentrated solutions of peroxides can result in skin and eye irritation, and in chemical burns. Angina, acute respiratory disease, and pneumonia have been reported among workers engaged in production and use of t-BP (*Emergency Response Guidebook*, 1987; Mohan, 1982). t-BP is not sufficiently stable to persist in the environment, and potential exposure of the general public is negligible (Radding, 1977).

t-BP was nominated for study by the National Cancer Institute because of its large-volume use, the potential for worker exposure during its manufacture and use, and as a representative organoperoxide, since it is one of the more stable members of this highly unstable class of chemicals. It also is more lipid-soluble than most organoperoxides and thus more likely to cross cell membranes to reach subcellular target sites. The toxicity of organoperoxides is of interest because, in addition to being commonly used industrial intermediates, these compounds generate free radicals. It is believed that free radicals generated intracellularly, as a result of chemical metabolism, may account for both the acute and chronic toxicity of many drugs and chemicals. However, free radicals are generated intracellularly as a result of metabolism of the respective chemicals by mixed-function oxidases or by other enzymatic activity (Kehrer, 1988). Little has been done to characterize the fate and toxicity of external sources of free radicals such as t-BP and related organoperoxides, other than to describe the acute effects resulting from contact with high concentrations.

Chronic studies with organoperoxides have been limited. Perbenzoate was shown to act as a promoter of carcinogenicity in mice initiated with other chemicals (Bock, 1975); in another study, benzoyl peroxide administered topically to mice and subcutaneously to mice and rats did not produce an increased incidence of tumors (Van Duuren, 1963; Sharratt, 1964). Thus, it was of interest to characterize both the fate of an organoperoxide in biological systems and intact animals, and the toxicity resulting from repeated exposure to a range of doses. The following report describes such studies, using t-BP as a model to determine the fate of an organoperoxide in rats, and the toxicity induced in 14-day and 13-week exposures in rats and mice. In addition, the toxicities of the degradation products of t-BP, which are t-butanol and benzoic acid, were evaluated in 14-day studies for comparative purposes. The gavage route was chosen to administer t-BP because the compound is unstable in food and is not normally inhaled, and because its absorption from skin was considered insufficient to permit administration of a toxic dose.

MATERIALS AND METHODS

Procurement and Characterization of t-Butyl Perbenzoate

t-BP used in these toxicity studies was manufactured by Penwalt Corporation (Lucidol Division, Buffalo, NY); the chemical was identified by NMR, infrared, and ultraviolet spectroscopy. Cumulative data derived from iodometric titration, elemental analysis, HPLC, and thin layer chromatography indicated a purity of > 98.8%. The bulk chemical was stored at room temperature, protected from light. Quantitative reanalyses were performed within a month prior to the initiation and completion of the 13-week studies; no degradation of the material was evident.

For the stability and disposition studies, [^{14}C]-t-butyl perbenzoate (Lot # 830107) was prepared by Pathfinder Laboratories, Inc. (St. Louis, MO). Labeled in the ring, the [^{14}C]-t-BP had a specific activity of 10 mCi/mmol. Unlabeled t-butyl perbenzoate (Lot # 32120J) was procured from Aldrich Chemical Co. (Milwaukee, WI). The purities of the unlabeled and labeled material were determined using 2 HPLC systems with a Waters Associates liquid chromatograph (Waters Chromatography, Milford, MA) equipped with 2 model 6000A pumps, a model 660 solvent programmer, a model U6K injector, and a model 440 ultraviolet detector operated at 254 nm. The flow rate was 2 ml/min. The first HPLC system that was used to determine the purity incorporated a linear solvent gradient beginning with CH_3CN:0.04M NH_4OAc; pH 6.5 (50:50) and ending with CH_3CN:0.04M NH_4OAc; pH 6.5 (95:5) in 10 minutes; the system used a Whatman Partisil® 10/ODS-3 column (Whatman, Inc., Clifton, NJ). Unlabeled t-BP was pure by HPLC analysis; radiochemical purity of the [^{14}C]-t-BP was 94 - 97%. The second HPLC system employed a linear gradient using a Lichrosorb® diol column (E. Merck, Rahway, NJ) with a mobile phase of heptane for 5 minutes and then heptane to heptane/n-propanol (95:5) in 5 minutes. The [^{14}C]-t-BP was 97% radiochemically pure; the unlabeled compound was essentially 100% pure.

Animals

F344/N rats and B6C3F$_1$ mice used in the 14-day and 13-week studies were produced under strict barrier conditions at Simonsen Laboratories, Inc. (Gilroy, CA). Animals were progeny of defined, microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats and mice were shipped to the study laboratory at 4 to 5 weeks of age, quarantined there for 11 days, and placed on study at approximately 6 weeks of age. Blood samples were collected and the sera analyzed for viral titers from 5 animals per sex and species at study start and termination in the 13-week studies. Data from 5 viral screens performed in rats and 12 viral screens performed in mice showed that there were no positive antibody titers (Boorman *et al.*, 1986; Rao *et al.*, 1989). For additional details on study design, see Table 1.

Adult male F344/N rats used in the disposition studies were purchased from Charles River Breeders (Kingston, NY). The rats were examined for diseases and abnormalities upon arrival and quarantined for 2 weeks before being used in a study. Animals were fed Certified Purina Rat Chow and water, *ad libitum*, for the duration of the studies. Food and water were withheld 15 hours prior to dose administration. Animals were transferred to glass metabolism chambers the day prior to being used in an experiment.

Stability Studies

Stability studies were performed with ^{14}C-labeled t-BP in corn oil at concentrations of 3 and 30 mg/ml, and in various biological media at concentrations of 1.1, 0.11, or 0.011 mg/ml. Biological media studied included 40 mg/ml BSA in buffered saline; Sorensen's buffer, 0.067M (pH 7.4); rat serum; whole blood; 20% stomach contents in Sorensen's buffer (pH 4.1); HEPES buffer (pH 7.4), with and without 5 mM glutathione; and liver microsomes and soluble fractions, with and without 5 mM glutathione. [^{14}C]-t-BP solutions in biological media were incubated at 37°C. Aliquots of 1 ml were withdrawn at 0, 5, 15, 30, and 60 minutes and extracted twice with ether. The pH of the sample was adjusted to 2, and the sample was extracted twice with ether. Radioactivity in the extracts was determined by scintillation spectrometry. The samples then were concentrated and reconstituted to 100-300 µl in ethanol and analyzed by HPLC.

t-BP/corn oil solutions were held at room temperature for for 0, 1, 2, or 24 hours. Aliquots of 10 µl were diluted with 60 µl of hexane and analyzed by HPLC. Initial conditions for the analysis were 100% hexane for 10 minutes followed by a 5-minute gradient of 100% hexane to 70:30 of hexane:hexane/ethanol (95:5). Analyses were performed using a Lichrosorb® diol column with a flow rate of 2 ml/min. Incubations (15 min) of 1.1 mg t-BP in 1 ml of liver microsomes, prepared from rats, were performed to analyze t-butanol content. GC/FID analysis was performed by direct injection of the incubation mixture onto an 80/100 Carbopak® column (Supelco, Bellefont, PA) at 120°C; nitrogen was used as the carrier gas, at a flow rate of 20 ml/min.

The stability of t-BP on isolated rat skin was assessed by direct application of t-BP solutions containing 0.1, 1, and 10 mg t-BP/20 µl ether, and 1.6, 2.0, and 2.3 x 10^6 DPM/20 µl ether, respectively. Skin was obtained from rats anesthetized with Ketamine®/Xylazine® by intraperitoneal injection. Their backs and sides were shaved; the animals were killed; and portions of the shaved skin were dissected into 2 cm^2 sections. Skin sections were placed in Petri dishes containing moist paper towels kept at 37°C, and 20 µl of each t-BP solution described above was placed on a 1 cm^2 area on each of 2 pieces of skin. The skin sections were kept at 37°C in a covered glass container with a small piece of moist paper towel. Skin samples receiving each of the 3 concentrations of t-BP were withdrawn and analyzed after 1 hour and after 24 hours. Each skin piece was rinsed with 5 ml of ether and 2 ml of ethanol, then sonicated in 20 ml of ether for 5 minutes. An aliquot of the extract was analyzed by HPLC using a Partisil® 10 ODS-3 column developed with a 20-minute linear gradient from 0.01M NH_4OAc (pH 4) to CH_3CN:0.01M NH_4OAc, pH 4 (98:2).

Disposition Studies

Distribution and excretion studies were performed following intravenous and dermal dose administration. Intravenous doses were administered in a tail vein and consisted of a dose volume of 1 µl/gram body weight Sorensen's buffer containing 4% rat serum albumin (w/v) and t-BP to obtain a target concentration level of 4 mg/kg. Dermal doses were prepared from ^{14}C-labeled and unlabeled material dissolved in ether to give target application doses of 0.38, 3.4, and 39 mg/kg t-BP. Dermal dosing solutions of 20 µl were applied to a 1 cm^2 shaved area on the backs of anesthetized animals. The area was secured using a 4 x 4 cm^2 square of adhesive backed foam with a 3 cm^2 square hole cut from the center, placed around the dosed area, and secured with

Superglue® (Loctite Corp., Cleveland, OH). A piece of hard-backed waxed paper was placed over the hole and secured with adhesive tape and Superglue®. Elastic adhesive bandage (Elastoplast®, Beirsdorf, Inc., South Norwalk, CT) then was placed over the entire area and glued around the edges to the skin.

Three animals receiving dermal applications at each dose level were placed in glass metabolism cages. Urine and feces were collected separately. Urine was collected at 2, 4, 6, 8, and 24 hours, and feces at 8 and 24 hours. Volatile organics and expired CO_2 were collected by drawing air from the metabolism cage at 200 - 500 ml/min, through an ethanol trap at 0°C, and through a series of 2 traps each containing 400 ml of 1N NaOH. Blood was collected by cardiac puncture at the end of the experiment (esterase activity was inhibited by addition of 12 mM physostigmine). A portion of each blood sample was separated into plasma and packed RBCs by centrifugation. Breath trap solutions were stored at room temperature. Blood was stored in the dark at 4°C until analyzed. All remaining samples were stored in the dark at -20°C.

All animals were killed by an overdose of Ketamine®/Xylazine® adminstered intravenously. Samples of all major tissues plus possible target tissues were taken for analysis of t-BP-derived ^{14}C. Plasma and urine analyses were performed to determine total radioactivity. Duplicate aliquots of plasma (0.1-0.2 ml) and urine (0.5 ml) were added to 10 ml of Scintiverse E® (Fisher Chemical Co., Pittsburgh, PA). Water or methanol was added as needed to obtain homogenous samples for scintillation counting. Feces and large tissues were homogenized in water. Entire small tissues and aliquots of the homogenates and blood were combusted in a Packard Model 306 oxidizer (Packard Instrument Co., Downers Grove, IL). Combusted samples were stored overnight in the dark before scintillation counting.

14-Day Study Design

Since t-BP degrades on contact with most biological media, it was of interest to determine if any toxicity observed on administration of t-BP was due to the parent compound or the degradation products. Studies were designed to evaluate the toxicity of equimolar doses of the parent compound and of its degradation products, t-butanol and benzoic acid. Doses chosen for t-BP were 70, 140, 278, 556, and 1112 mg/kg for both sexes of rats and mice. The high dose represents approximately one-fourth the oral LD_{50} of this compound for rats and one-half the LD_{50} for mice. Equimolar doses of t-butanol were 30, 60, 120, 242, and 484 mg/kg; equimolar doses of benzoic acid were 40, 80, 160, 321, and 642 mg/kg. All chemicals were administered in corn oil, 5 ml/kg, to groups of 5 animals in daily doses, 5 days per week. Details of clinical and pathology examinations are outlined in Table 1. Organs weighed at the end of the studies included thymus, heart, lung, esophagus, stomach, liver, kidney, brain, urinary bladder, and testis.

13-Week Study Design

Groups of 10 rats and 10 mice of each sex were given t-BP by gavage in deionized water, 5 ml/kg, at levels of 0, 30, 60, 125, 250, and 500 mg/kg body weight. Systemic toxicity observed in administration of benzoic acid, and the lack of comparable systemic toxicity induced by t-BP in 14-day studies, led to speculation that benzoic acid, insoluble in corn oil, was absorbed directly from the stomach as a bolus, while t-BP was absorbed more slowly from the small intestine as the corn oil was digested. To more closely mimic any possible human exposure expected to result in

absorption of t-BP and its degradation products from the stomach, doses in the 13-week studies were administered in water. The selected high dose represents the highest dose of t-BP that can be prepared as a homogeneous suspension in water.

Details of clinical examinations and pathology performed are outlined in Table 1. Animals surviving to the end of the study were killed with CO_2. Complete necropsies were performed on all animals; organs and tissues were examined for gross lesions. Tissues were preserved in 10% neutral buffered formalin and routinely processed for preparation of histologic sections for microscopic examination. Tissues for microscopic evaluation were trimmed to a maximum of 3 mm. Following dehydration and embedding, tissues were sectioned at approximately 5 microns, stained with hematoxylin and eosin, and examined microscopically. The specific tissues examined are listed in Table 1. Organs weighed at the end of the study include brain, forestomach, glandular stomach, spleen, right kidney, testis, thymus, liver, heart, and lung.

Upon completion of the histologic evaluation by the laboratory pathologist, slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. Slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory for quality assessment; the results were reviewed and evaluated by NTP's Pathology Working Group (PWG). The final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). At the end of the 13-week study, blood smears were prepared from mice for erythrocyte micronuclei determinations. Blood smears were prepared (unstained) and fixed in 100% methanol.

TABLE 1 Experimental Design and Materials and Methods in the 14-Day and 13-Week Gavage Studies of t-Butyl Perbenzoate

Study Laboratory	Battelle Columbus Laboratories Columbus, OH
Study Dates	March, 1985 -- July, 1988
Strain and Species	F344/N rats, $B6C3F_1$ mice
Animal Source	Simonsen Laboratories, Inc , Gilroy, CA
Chemical Source	Penwalt Corporation, Lucidol Division, Buffalo, NY
Size of Study Groups	14-Day Studies 5 males and 5 females of each species per dose group Rats and mice were housed 5 per cage 13-Week Studies 10 males and 10 females of each species per dose group Rats were housed 5 per cage, mice were housed individually
Doses	14-Day Studies 0, 70, 140, 278, 556, 1112 mg t-butyl perbenzoate, 30, 60, 120, 242, 484 mg t-butanol, or 40, 80, 160, 321, 642 mg benzoic acid per kg body weight in corn oil, by gavage 13-Week Studies 0, 30, 60, 125, 250, 500 mg t-butyl perbenzoate per kg body weight in deionized water by gavage
Method of Animal Distribution	Animals randomized and assigned to study groups using a consecutive identification numbering system
Diet	NIH 07 pelleted feed and water, *ad libitum*
Animal Room Environment	Temp 68-75°F, relative humidity 35-65%, fluorescent light 12 h/d, 12-15 room air changes/h

TABLE 1 Experimental Design and Materials and Methods in the 14-Day and 13-Week Gavage Studies of t-Butyl Perbenzoate (continued)

Time Held Before Study	14-Day and 13-Week Studies Rats 11 d, Mice 11 d
Age When Placed on Study	14-Day and 13-Week Studies 6 wks
Duration of Dosing	14-Day Studies 1 x d for 5 d/wk for total of 12 doses over 16 days 13-Week Studies 1 x d for 5 d/wk with 2 consecutive doses prior to necropsy, last dose within 24 hours of necropsy
Age When Killed	14-Day Studies 8 wks 13-Week Studies 19 wks
Type and Frequency of Observation	14-Day Studies Observed 2 x d for mortality/moribundity, 1 x wk for clinical signs of toxicity, weighed initially, on day 8, and at necropsy 13 Week Studies Observed 2 x d for mortality/moribundity, 1 x wk for clinical signs of toxicity, weighed initially, weekly, and at necropsy

Necropsy and Histologic Examinations
Necropsy performed on all animals, the following tissues were examined microscopically

14-Day Studies -- for mice, complete examination of controls and high dose animals, stomach, esophagus, urinary bladder, and right kidney examined at all lower doses, for rats, complete examination of controls and 3 highest doses of benzoic acid, and highest dose groups for t-BP and t-butanol, stomach, esophagus, urinary bladder, and right kidney examined in all other dose groups

13-week studies -- for mice and rats, complete examination of all controls and high dose animals, forestomach and gross lesions examined at lower dose levels Complete histopathologic examination included the following tissues gross lesions and tissue masses (regional lymph nodes), blood smear, mandibular and mesenteric lymph node, salivary gland, sternebrae, femur, or vertebrae (including marrow), thyroid, parathyroids, liver, gall bladder (mice), heart, esophagus, stomach (glandular and forestomach), brain (frontal cortex, basal ganglia, parieteal cortex and thalamus, cerebellum and pons), thymus, pancreas, trachea, small intestine (duodenum, jejunum, ileum), large intestine (cecum, colon, and rectum), prostate, testes/epididymus, uterus, ovaries, preputial and clitoral glands, lungs and mainstem bronchi, nasal cavity and turbinates, spleen, kidneys, adrenals, urinary bladder, pituitary, spinal cord and sciatic nerve (if neurologic symptoms present), eyes (if grossly abnormal), mammary gland (to include surface skin)

Statistical Methods

The significance of differences between dosed and control group means was assessed using multiple comparison procedures designed to protect against false positive inferences. Either Dunn's test or Williams' modification of Shirley's multiple comparisons procedure was applied based on the occurrence of a dose-related response in the data (Dunn, 1964; Shirley, 1977; and Williams, 1986). Shirley's test is designed to detect treatment-related differences when the response to treatment consistently increases or decreases as the dose level increases. Dunn's test is appropriate if the departure from monotonicity is severe. If the p value from Jonckheere's test (Hollander and Wolfe, 1973) for a dose-related trend was greater than or equal to 0.10, Dunn's test was used rather than Shirley's test. The outlier test of Dixon and Massey (1951) was employed to detect extreme values. Details of further statistical methods are given in table footnotes.

Quality Assurance

The t-BP studies were performed in compliance with FDA Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of Battelle Columbus Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies. The NTP monitored operations of the Quality Assurance Unit.

RESULTS

Stability and Disposition Studies

t-BP containing a ^{14}C label in the benzoate portion of the molecule was used in all studies of the fate and stability of this compound. Through HPLC analysis, t-BP was determined to be stable for 24 hours in corn oil at room temperature, and 97% stable for 1 hour at room temperature in a solution of 4% rat serum albumin in Sorensen's buffer, pH 7.4. This stability was sufficient to permit preparation and administration of dose solutions for oral gavage in corn oil, and i.v. administration in buffered albumin. These preparations were used in the respective *in vivo* studies. t-BP was stable in HEPES buffer, pH 7.4, for up to an hour at 37°C; the addition of glutathione to the HEPES buffer, however, resulted in concentration and time dependent degradation. t-BP solutions of 0.011, 0.11, and 1.1 mg/ml in HEPES buffer, pH 7.4, containing 5 mM glutathione, were degraded by 22, 18, and 10% in 15 minutes, respectively.

In vitro studies established that t-BP was not stable in rat blood at 37°C. At all concentrations studied (0.011 to 16 mg/ml), more than 50% of the t-BP degraded within 15 minutes after addition to blood. t-BP appeared to be even less stable in experiments with human blood than in those with rat blood. Half-lives of 4 mg/ml in rat and human blood were estimated to be 10.4 and 4.0 minutes, respectively. Degradation or metabolism of t-BP in microsomal or soluble enzyme preparations from rat liver was extremely rapid; less than 1% of concentrations of 0.011, 0.11 or 1.1 mg/ml could be recovered as parent compound from incubations with either fraction after 15 minutes. Benzoic acid and t-butanol made up 93% of the major degradation and/or metabolic products. t-BP was stable in Sorensen's buffer, pH 4.1, for 1 hour, but degraded in a 20% suspension of stomach contents in this buffer in a concentration-dependent fashion. t-BP concentrations of 1.1, 0.11, and 0.011 mg/ml degraded by 0, 31, and 74%, respectively, in 1 hour at 37°C in a suspension of stomach contents.

Human exposure to t-BP is anticipated to be primarily by the dermal route because of its instability in the environment and pattern of use. t-BP's stability on skin and its distribution following dermal administration were determined in the rat. Tissue distribution of t-BP following i.v. administration was determined, for comparative purposes, to simulate 100% absorption of intact chemical.

The stability of t-BP on isolated skin at 37°C is shown in Table 2. After 1 hour, both the fraction of t-BP that could be removed from the skin by rinsing, and the proportion of this material remaining as parent compound, were greater at 10 mg/cm^2 than at lower concentrations. It appeared that t-BP was quite stable on skin for 1 hour at a concentration of 10 mg/cm^2, but degraded by approximately 34% and 48% at 1.0 and 0.1 mg/cm^2, respectively. Absorption or binding of t-BP increased as the doses decreased and accounted for over 30% of the lower dose within 1 hour. At 24 hours, the amount of t-BP bound or absorbed to skin accounted for approximately 70% of all doses; the amount of parent compound detected in the rinse was minimal. The major decomposition product of t-BP detected in the rinse or extract of all skin samples in this study was benzoic acid.

TABLE 2 Stability of t-Butyl Perbenzoate on Isolated Rat Skin[a]

Amount of t-BP/cm^2 (mg)[b]	Time Exposed to Skin (hr)	% Total Rinsed from Skin[c]	% Rinse as t-BP[d]	% Total in Skin after rinsing[e]	% Total (Rinse + Skin) Recovered
10 0	1	106[f]	100	2	107
1 0	1	73	66	10	83
0 1	1	72	52	31	100
10 0	24	8	4	73	81
1 0	24	7	0	77	83
0 1	24	7	0	70	76

a Shaved skin isolated from backs of adult male rats and held in humid chamber at 37°C
b [^{14}C]-t-BP applied in 20 ml ether to 1 cm^2 isolated rat skin
c Skin was extracted sequentially first with ethanol, then ether
d Determined by HPLC analysis
e Determined by digestion and scintillation counting
f Data represent an average of 2 determinations

In intact animals, the degree of absorption of a dermal dose of t-BP apparently was not affected by the size of the dose administered in the range studied (Table 3). These data indicate that approximately 13% to 14% of the administered radioactivity was absorbed from skin and eliminated in urine within the first 24 hours after dosing Elimination in feces and expired breath (data not shown) was minimal; combined, they never accounted for more than 1% of the radioactivity administered. When a dose of 3.7 mg/kg t-BP was administered i.v. to simulate 100% absorption, most of the dose was excreted in urine within 8 hours (Table 4). Excretion in feces was minimal, and less than 0.1% of the dose was eliminated in breath (data not shown).

TABLE 3 Cumulative Excretion of ^{14}C after Dermal Application of [^{14}C]-t-Butyl Perbenzoate to F344/N Rats[a]

Dose Applied (mg/kg)	0 377		3 37		38 96	
	Urine	Feces	Urine	Feces	Urine	Feces
Time (hr)						
2	0 67 ± 0 58[b]		1 01 ± 1 10		0 16 ± 0 14	
4	3 76 ± 0 42		2 23 ± 1 35		0 34 ± 0 42	
6	4 81 ± 1 03		4 48 ± 1 72		2 10 ± 1 84	
8	5 29 ± 1 84	0 00 ± 0 00	5 61 ± 3 29	0 30 ± 0 27	2 87 ± 1 32	0 17 ± 0 24
24	13 10 ± 2 7	0 59 ± 0 46	12 70 ± 5 8	0 38 ± 0 30	14 30 ± 1 31	0 39 ± 0 45

a [^{14}c]-t-BP applied in 20 ml ethyl ether to 1 cm^2 of shaved back area of anesthetized rats Application site was protected from grooming by a nonocclusive cover
b Percent Dose ± Standard Deviation

An estimate of dermal absorption based on a comparison of data in Tables 3 and 4, assuming that elimination is not altered by the route of administration, indicated that approximately 16% of each of the dermal doses was absorbed. The nature of the material absorbed (that is, parent or degradation products of t-BP) could not be determined due to the instability of t-BP on skin and in blood.

The distribution of t-BP-derived radioactivity following i.v. or dermal administration is shown in Table 5. As could be inferred from data presented in Tables 3 and 4, the levels of radioactivity remaining in tissues 24 hours after administration were low. Further, radioactivity retained in tissues 24 hours after dosing was relatively evenly distributed throughout the tissues; in most

instances, tissue/blood ratios did not vary from unity by a factor of more than 2 or 3. Skin was an exception in the dermal studies, in that the concentration of t-BP-derived radioactivity in skin was usually at least 10 times higher than that in blood. This was not seen with i.v. administration and probably represents some cross contamination from the dose site in the dermal studies. Concentrations in the intestines were high, but these data were too variable to permit speculation as to the significance of this observation. Radioactivity remaining in the tissues at the dose site in the dermal studies was quite high. Retention of radioactivity in these tissues is consistent with the binding of t-BP and/or its degradation products to skin observed in the *in vitro* studies described in Table 2. It is interesting to note that retention at the dose site is proportional to the dose administered.

TABLE 4 Cumulative Excretion of Total ^{14}C after Intravenous Administration of 3.7 mg/kg of [^{14}C]-t-Butyl Perbenzoate to Male F344/N Rats[a]

Time (hr)	Urine	Feces	Total
2	56 1 ± 22 2		56 1 ± 22 2
4	76 6 ± 7 2		76 6 ± 7 2
6	78 5 ± 6 8		78 5 ± 6 8
8	81 4 ± 6 4		81 4 ± 6 4
24	85 0 ± 4 3	1 0 ± 1 1	86 0 ± 4 4
48	87 2 ± 0 0	1 1 ± 1 2	88 3 ± 1 2
72	89 4 ± 3 9	1 2 ± 1 2	90 6 ± 4 1

a I V administration into a tail vein in a solution of 1% ethanol in Sorensen's buffer, pH 7 4, containing 40 mg rat serum albumin/ml
b Percent Dose ± Standard Deviation

TABLE 5 Concentration of t-Butyl Perbenzoate-Derived ^{14}C in Tissues 24 Hours after Intravenous[a] or Dermal[b] Administration of [^{14}C]-t-BP

	ng - eq/g [c]			
DOSE (mg/kg)	3 9 (i v)	0 377 (Dermal)	3 37 (Dermal)	39 0 (Dermal)
Blood	52 ± 4	1 0 ± 0 13	10 9 ± 0 6	141 ± 18
Skin	50 ± 28	14 0 ± 2	120 ± 40	860 ± 290
Stomach	13 ± 8	2 9 ± 2 3	49 ± 36	210 ± 50
Liver	32 ± 3	2 3 ± 1 5	32 ± 17	140 ± 8
Lung	35 ± 6	1 1 ± 0 4	10 ± 6 5	86 ± 13
Heart	22 ± 6	0 94 ± 0 32	3 9 ± 2 5	52 ± 4
Kidneys	35 ± 17	4 5 ± 0 9	33 ± 12	540 ± 132
Adipose	18 ± 7	1 4 ± 0 4	4 8 ± 1 8	720 ± 770
Adrenals	37 ± 4 6	0 9 ± 0 05	5 0 ± 2 9	99 ± 21
Small Intestines, full	536 ± 783	8 9 ± 12	29 ± 42	400 ± 430
Small Intestines, clean	47 ± 22	1 8 ± 1 0	63 ± 42	880 ± 2,100
Large Intestines, full	69 ± 17	6 6 ± 1 8	59 ± 60	1,800 ± 2,500
Large Intestines, clean	22 ± 1	1 1 ± 0 1	6 5 ± 4 0	4,200 ± 7,100
Cecum	79 ± 34	7 3 ± 3 2	41 ± 44	730 ± 320
Muscle	8 ± 5 2	0 48 ± 0 08	3 1 ± 3 4	380 ± 180
Brain	11 ± 3	0 16 ± 0 01	1 3 ± 0 4	120 ± 10
Spleen	17 ± 2	0 50 ± 0 01	4 0 ± 1 1	41 ± 6
Site of Dose				
Muscle under Dose Site		1 1 ± 0 3	4 3 ± 3 1	110 ± 50
Subcutaneous Fat Under Dose Site		6 3 ± 3 4	20 ± 9	390 ± 170
Brown Fat Under Dose Site		16 ± 8	51 ± 14	1,700 ± 1,700
Dermis Under Dose Site		4,500 ± 4,300	5,400 ± 7,300	64,000 ± 170,000
Dose Site		1,900 ± 700	21,000 ± 13,000	240,000 ± 59,000

a I V dose administered as described in Table 4
b Dermal dose applied as described in Table 3
c All data represent average (±SD) ng t-BP equivalents per gram tissue obtained from at least 3 animals

14-Day Toxicity Studies in F344/N Rats

No deaths occurred among control rats, or among rats that received t-BP or t-butanol. Two male rats receiving benzoic acid died during the study (Table 6). One receiving 321 mg/kg was killed in a

TABLE 6 Survival and Weight Gain of F344/N Rats in the 14-Day Gavage Studies of t-Butyl Perbenzoate, t-Butanol, and Benzoic Acid

Dose Level (mg/kg)	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls(%)[c]
MALE					
t-Butyl Perbenzoate					
1112	5/5	102	153	51	100
556	5/5	100	165	65	108
278	5/5	102	169	67	110
140	5/5	103	173	70	113
70	5/5	104	173	69	113
t-Butanol					
484	5/5	100	168	68	110
242	5/5	105	176	71	115
120	5/5	101	170	69	111
60	5/5	100	169	69	110
30	5/5	99	170	71	111
Benzoic Acid					
642	5/5	102	167	65	109
321	4/5	102	166	64	108
160	4/5	100	161	61	105
80	5/5	104	175	71	114
40	5/5	102	169	67	110
Vehicle Control					
0	5/5	101	153	52	
FEMALE					
t-Butyl Perbenzoate					
1112	5/5	84	119	35	96
556	5/5	86	132	46	106
278	5/5	84	126	42	102
140	5/5	84	124	40	100
70	5/5	87	126	39	102
t-Butanol					
484	5/5	84	123	39	99
242	5/5	84	125	41	101
120	5/5	83	129	46	104
60	5/5	83	119	36	96
30	5/5	81	121	40	98
Benzoic Acid					
642	5/5	84	116	32	94
321	5/5	85	122	37	98
160	5/5	85	122	37	98
80	5/5	86	128	42	103
40	5/5	84	124	40	100
Vehicle Control					
0	5/5	85	124	39	

a Number surviving at 14 days/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/Control group mean) x 100.

moribund condition on day 6; the other, in the 160 mg/kg group, died on day 13. Food consumption by dosed males and females was comparable to or slightly higher than that of the control groups (data not shown). Initial and final mean body weights for rats are shown in Table 6. Body-weight gains of all treated male rats, except those in the high dose t-BP group, were greater than those of the control group. However, the low weight gain of the control group of male rats may account for these findings. Female rats receiving the highest dose of t-BP or benzoic acid gained less weight than controls, but the difference was not statistically significant.

During the in-life portion of this study, 1 male rat receiving 321 mg/kg of benzoic acid was observed to have labored respiration and was lethargic, leading to its moribund sacrifice. No treatment-related gross lesions were observed in rats at the end of the studies. At study termination, liver weights of female rats receiving 556 and 278 mg/kg t-BP were higher by as much as 20%, than those of controls; both these groups, and females in the high dose group (1112 mg/kg), had higher mean liver-to-body-weight ratios. Thymus weights and thymus-to-body-weight ratios of female rats in the highest dose t-BP group were about 20% lower than controls. Stomach was considered a possible target tissue for t-BP administrated by gavage, but the only increases in absolute and relative stomach weight (about 25%) were seen with male rats receiving the highest dose. Other variations in organ weights observed in male and female rats appeared neither remarkable nor dose-related. Histopathological examination revealed no lesions that were considered related to administration of t-BP, t-butanol, or benzoic acid.

13-Week Toxicity Studies in F344/N Rats

All treated and control male rats survived to the end of the study. One female in the 250 mg/kg group died during week 5 (Table 7); a control in the female study was removed because it was missexed. Food consumption was similar in all groups of the treated and control animals except

TABLE 7 Survival and Weight Gain of F344/N Rats in the 13-Week Gavage Studies of t-Butyl Perbenzoate

Dose Concentration (mg/kg)	Survival[a]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[c]
		Initial	Final	Change[b]	
MALE					
0	10/10	118	356	238	
30	10/10	120	369	249	103
60	10/10	117	358	241	101
125	10/10	117	359	242	101
250	10/10	120	357	237	100
500	10/10	117	336	219	94
FEMALE					
0	9/10	97	203	106	
30	10/10	98	198	100	98
60	10/10	98	195	97	96
125	10/10	98	196	98	96
250	9/10	99	201	102	99
500	10/10	97	186	89	92

[a] Number surviving at 13 weeks/number of animals per dose group.
[b] Mean weight change of the animals in each dose group.
[c] (Dosed group mean/Control group mean) x 100.

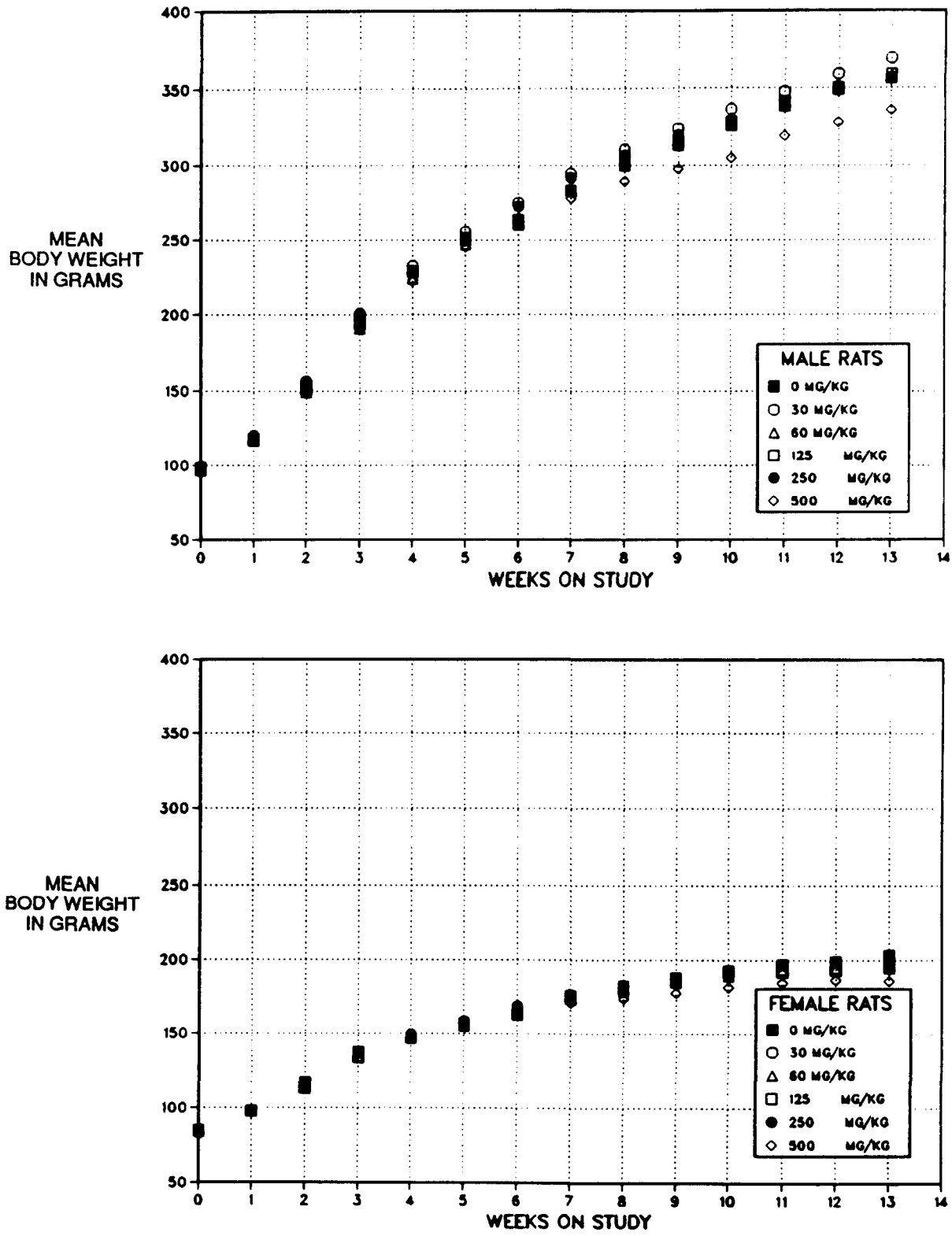


Figure 1 Body Weights of F344/N Rats Exposed to t-Butyl Perbenzoate by Gavage for 13-Weeks

in high dose female rats, whose food consumption was about 7% less than controls. Mean body weights are shown in Figure 1 and in Table 7. Body-weight gains of male and female rats in the highest dose groups were depressed after about week 7.

A variety of clinical observations were noted in both male and female rats during the course of the study, but none were attributed to administration of t-BP. Similarly, at necropsy, no apparent chemical-related gross lesions were observed in either sex of rats. Forestomach weights were increased in male rats receiving the 250 and 500 mg/kg doses and in female rats receiving 60 mg/kg and higher doses (Appendix A, Table A1). Weights of the glandular stomachs also were increased in both males and females, but the increases were largely restricted to high dose animals and the percent increase was smaller than observed in the forestomach. Other changes in organ weights included slightly decreased spleen weights in males and females receiving the high dose, and increased kidney weights in female rats receiving 250 mg/kg (Appendix A, Table A1).

Epithelial hyperplasia and inflammation were observed in the forestomach of dosed rats (Table 8). Dose-related increases in the incidence and severity of squamous epithelial hyperplasia were seen in male and female rats. Within the hyperplastic epithelium there was increased mitotic activity of the basal cell layer, rete peg-like downgrowths of hyperplastic cells, and variable hyperkeratosis, which appeared to increase in severity with the degree of hyperplasia present (Plates 1-3). Inflammatory cell infiltration also was evident in the forestomach of rats in the higher dose groups (Table 8). These inflammatory changes included leukocytic exocytosis with neutrophil aggregates within the hyperkeratotic layer, as well as within intraepithelial clefts and vesicles (Plate 4); congestion of subepithelial capillaries, perivascular edema, and microhemorrhages were components of inflammation in some rats.

TABLE 8 Histopathologic Lesions in F344/N Rats in the 13-Week Gavage Studies of t-Butyl Perbenzoate

	t-BP (mg/kg)					
	0	30	60	125	250	500
MALE						
Forestomach						
Epithelial hyperplasia	0/10	2/10 (1.0)[a]	4/10 (1.0)	5/10 (1.0)	10/10 (1.4)	10/10 (1.7)
Inflammation	0/10	0/10	0/10	0/10	5/10 (1.2)	8/10 (1.0)
FEMALE						
Forestomach						
Epithelial hyperplasia	0/9	0/10	2/10 (1.0)	5/10 (1.0)	9/10 (1.2)	10/10 (1.8)
Inflammation	0/9	0/10	0/10	1/10 (1.0)	2/10 (1.0)	3/10 (1.3)

[a] Average severity score based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on the number of animals with lesions.

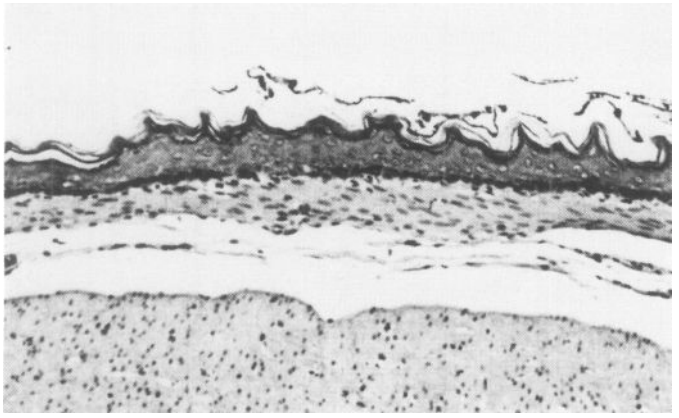


Plate 1
Forestomach of a control male rat from the 13-week t-butylperbenzoate studies.

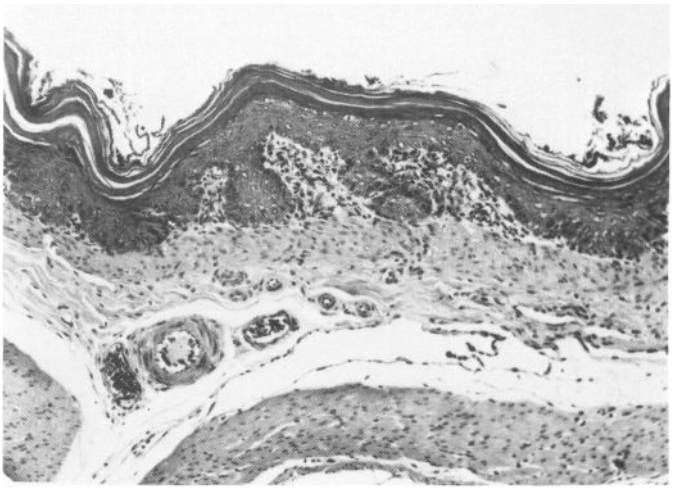


Plate 2
Hyperplasia, hyperkeratosis, and submucosal inflammation of the forestomach of a 250 mg/kg dose male rat from the 13-week t-butylperbenzoate studies.

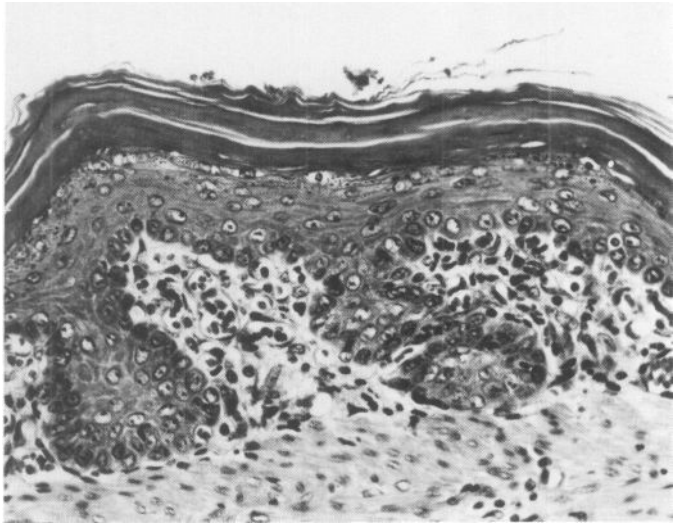


Plate 3
Higher magnification of plate 2 details downgrowths of hyperplastic epithelium, with subepithelial capillary dilatation and inflammatory cell infiltration.

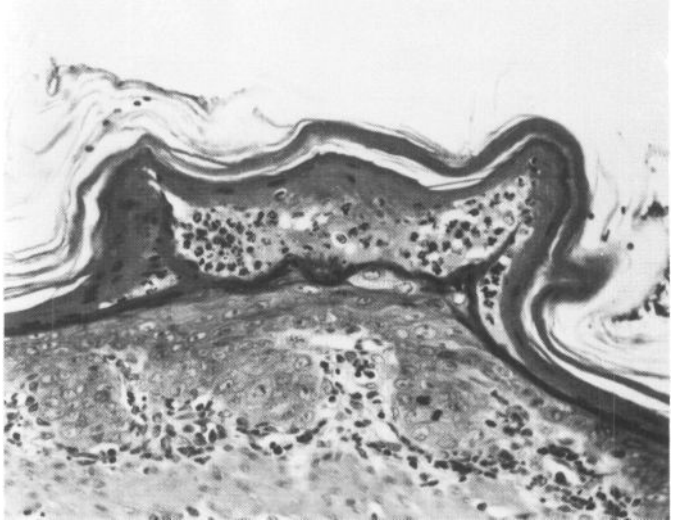


Plate 4
Forestomach of a 250 mg/kg dose male rat from the 13-week t-butylperbenzoate studies, demonstrating intra-epithelial clefting due to neutrophilic exocytosis, hyperplasia with mitoses, and subepithelial inflammation.

14-Day Toxicity Studies in B6C3F$_1$ Mice

No male mice receiving t-BP or t-butanol died during the study. However, 3 males receiving the highest dose of benzoic acid died during week 1 (Table 9). One female mouse receiving the highest

TABLE 9 Survival and Weight Gain of B6C3F$_1$ Mice in the 14-Day Gavage Studies of t-Butyl Perbenzoate, t-Butanol, and Benzoic Acid

Dose Level (mg/kg)	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]
MALE					
t-Butyl Perbenzoate					
1112	5/5	21	25	4	100
556	5/5	22	25	3	100
278	5/5	21	25	4	100
140	5/5	21	26	5	104
70	5/5	22	25	3	100
t-Butanol					
484	5/5	21	24	3	96
242	5/5	21	25	4	100
120	5/5	21	25	4	100
60	5/5	21	25	4	100
30	5/5	21	24	3	96
Benzoic Acid					
642	2/5	21	25	4	100
321	5/5	21	24	3	96
160	5/5	21	24	3	96
80	5/5	23	24	2	96
40	5/5	21	24	3	96
Vehicle Control					
0	5/5	22	25	3	
FEMALE					
t-Butyl Perbenzoate					
1112	4/5	18	21	3	100
556	5/5	18	21	3	100
278	5/5	18	21	3	100
140	5/5	18	21	3	100
70	5/5	18	21	3	100
t-Butanol					
484	4/5	18	21	3	100
242	5/5	17	21	4	100
120	5/5	18	21	3	100
60	5/5	18	21	3	100
30	5/5	18	21	3	100
Benzoic Acid					
642	4/5	18	20	2	95
321	5/5	18	21	3	100
160	5/5	18	21	3	100
80	5/5	18	21	3	100
40	5/5	18	21	3	100
Vehicle Control					
0	5/5	17	21	4	

a Number surviving at 14 days/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/Control group mean) x 100.

dose of t-BP was killed in moribund condition on study day 3; another female, receiving the highest dose of t-butanol, died on day 4; and a female receiving the highest dose of benzoic acid was killed in moribund condition midway through the study (Table 9). Food consumption was somewhat lower in groups of mice of both sexes receiving t-butanol and benzoic acid, but differences were not significant in either sex compared to controls. Weight gains of groups of treated male and female mice were generally similar to those of controls (Table 9).

Mice dosed with t-BP or t-butanol showed no clinical signs considered to be related to administration of the chemical. However, male and female mice receiving the 642 mg/kg dose of benzoic acid exhibited rough hair coats, labored breathing, hunched posture, salivation, and enlarged abdomen. As indicated above, 3 of 5 male mice in this dose group died during week 1.

The only lesion observed at necropsy that was considered possibly treatment-related was a single pigmented focus in the stomach of a female mouse receiving the highest dose of t-BP. Similarly, little or no effect of administration of these 3 chemicals was observed on most absolute or relative organ weights. However, stomach weights were increased by as much as 2-fold, in a dose-dependent manner, in the 3 highest dose groups of male mice and in the 2 highest dose groups of female mice. Increases in stomach weights also were observed in mice receiving t-butanol, but these were not dose-related; no increases in stomach weights were observed in mice receiving benzoic acid.

Lesions considered related to administration of t-BP were observed only in the forestomach (Table 10). These lesions included hyperplasia, ulceration, and acute inflammation of the forestomach mucosa. Hyperplasia was the most common lesion and was characterized by increased cellularity and basophillia of the epithelium, with variable degrees of hyperkeratosis. Hyperplasia was observed in the forestomachs of all mice that received the 2 highest doses and which survived the 14-day study, and in 3 of 5 females receiving the third highest dose, 278 mg/kg. A shallow ulcer was observed in the forestomach of 1 female mouse receiving the highest dose, corresponding to the gross lesion mentioned above.

TABLE 10 Histopathologic Lesions in B6C3F$_1$ Mice in the 14-day Gavage Studies of t-Butyl Perbenzoate[a]

	t-BP (mg/kg)					
	0	70	140	278	556	1112
Site/Lesion						
MALE						
Forestomach						
Epithelial hyperplasia	0/5	0/5	0/5	0/5	5/5 (2.0)[b]	5/5 (3.0)
Acute Inflammation	0/5	0/5	0/5	0/5	0/5	1/5 (1.0)
Site/Lesion						
FEMALE						
Forestomach						
Epithelial hyperplasia	0/5	0/5	0/5	3/5 (1.0)	5/5 (1.8)	4/5 (3.0)[c]
Acute Inflammation	0/5	0/5	0/5	0/5	0/5	1/5 (1.0)
Ulceration	0/5	0/5	0/5	0/5	0/5	1/5 (3.0)

a All surviving animals were examined in each group.
b Average severity score based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on tissues with lesions.
c One animal in this group was killed in moribund condition prior to the end of the study.

13-Week Toxicity Studies in B6C3F$_1$ Mice

Two mice died during the study. One control male died on day 4 of apparent gavage error, and a female in the 250 mg/kg dose group died on day 3. Mean diet consumption and body-weight gains by all dosed groups of mice were similar to those of the respective control groups (Figure 2 and Table 11). Clinical observations during the course of the study and gross observations at necropsy revealed few signs of toxicity or macroscopic lesions related to t-BP administration.

TABLE 11 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Gavage Studies of t-Butyl Perbenzoate

Dose Concentration (mg/kg)	Survival[a]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[c]
		Initial	Final	Change[b]	
MALE					
0	9/10	23	34	11	
30	10/10	23	35	12	103
60	10/10	23	34	11	100
125	10/10	22	34	12	100
250	10/10	23	34	11	100
500	10/10	23	33	10	97
FEMALE					
0	10/10	19	31	11	
30	10/10	20	29	9	93
60	10/10	19	30	11	97
125	10/10	20	30	10	97
250	9/10	19	28	9	90
500	10/10	21	30	9	97

a Number surviving at 13 weeks/number of animals per dose group
b Mean weight change of the animals in each dose group
c (Dosed group mean/Control group mean) x 100

Evidence of t-BP toxicity in mice was limited to increased stomach weights and lesions in the stomachs of dosed animals. Forestomach weights of both sexes receiving 250 and 500 mg/kg were increased by 50% or more (Appendix A, Table A2). Additionally, glandular stomach weights of female mice in the 500 mg/kg group, and the glandular stomach-to-body-weight ratios of female mice in the 250 mg/kg group were significantly increased compared to controls. Other changes in organ weights and organ-to-body-weight ratios were not considered related to chemical toxicity. Histopathological examination of the forestomachs revealed compound-related hyperplasia of the stratified squamous epithelium in male and female mice. In males, all dose groups were affected, although hyperplasia in a single animal in the 30 mg/kg group was minimal and not clearly compound-related. In females, the lesion was seen in the 60 mg/kg and higher dose groups (Table 12). This lesion increased in both frequency and severity as the dose increased. Hyperplasia was characterized by increased cellularity and basophilia of the squamous epithelium, with hyperkeratosis that also appeared to increase with dose.

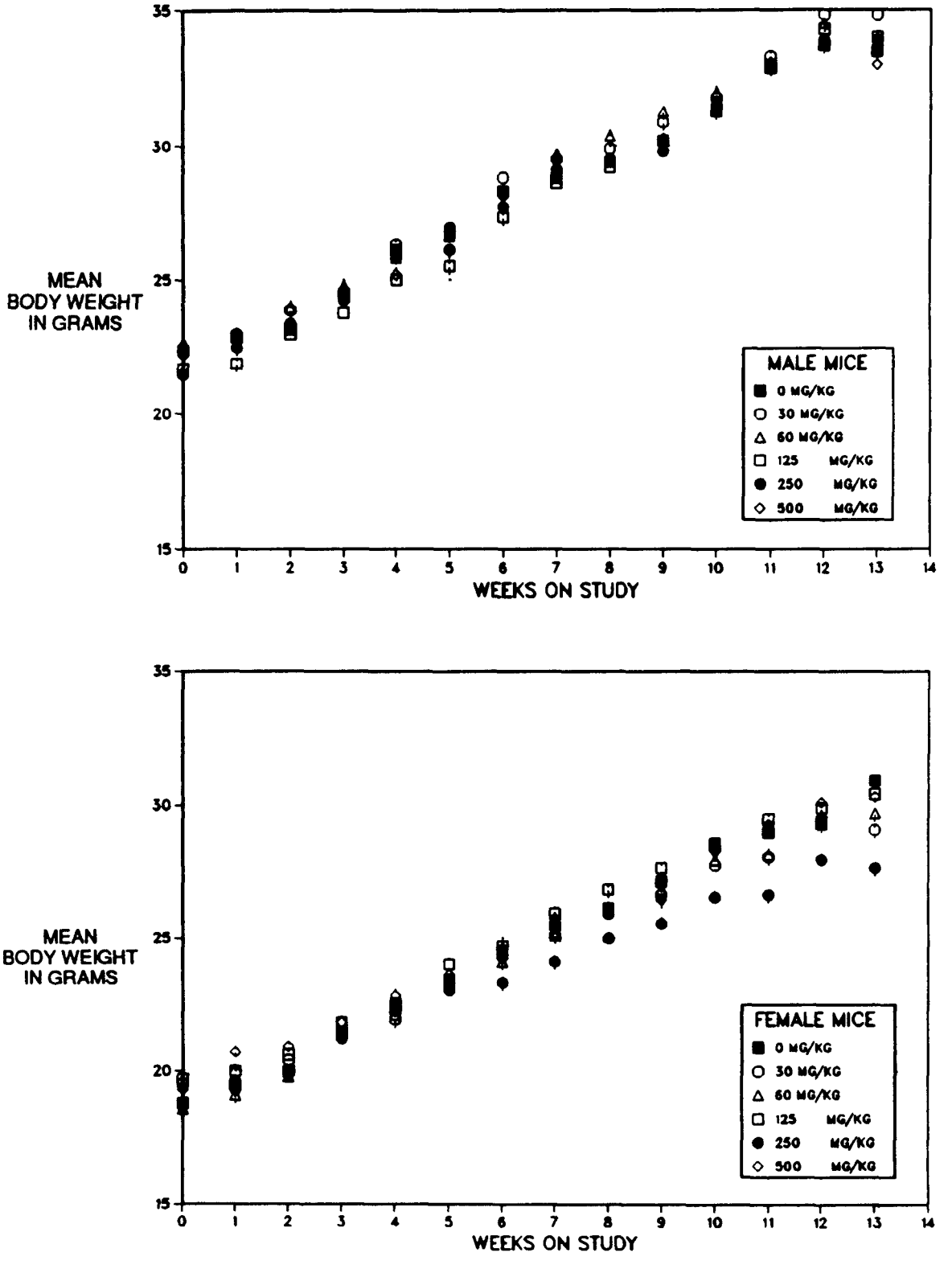


Figure 2 Body Weights of B6C3F$_1$ Mice Exposed to t-Butyl Perbenzoate by Gavage for 13-Weeks

TABLE 12 Histopathologic Lesions in B6C3F$_1$ Mice in the 13-Week Gavage Studies of t-Butyl Perbenzoate

	t-BP (mg/kg)					
	0	30	60	125	250	500
MALE						
Forestomach						
Epithelial Hyperplasia	0/10	1/10 (1.0)[a]	4/10 (1.0)	10/10 (1.0)	10/10(1.0)	10/10 (2.0)
FEMALE						
Forestomach						
Epithelial Hyperplasia	0/10	0/10	5/10 (1.0)	10/10 (1.1)	10/10 (1.1)	10/10 (1.7)

[a] Average severity score based on a scale of 1 to 4 1 = minimal, 2 = mild, 3 = moderate, 4 = marked Scores are averages based on the number of animals with lesions

Genetic Toxicology

t-BP (0.300-333 μg/plate) was mutagenic in *Salmonella typhimurium* strains TA100, TA1537, and TA98 when tested in a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9; no mutagenic response was observed in strain TA1535 with or without S9 (Mortelmans *et al.*, 1986; Appendix B, Table B1). In cytogenetic tests with Chinese hamster ovary (CHO) cells, t-BP induced sister-chromatid exchanges (SCE) within a concentration range of 1.0 to 5.0 μg/ml in the absence of S9 activation; no induction of SCE was observed in the presence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Appendix B, Table B2). t-BP was an effective inducer of chromosomal aberrations in CHO cells with and without S9 (Appendix B, Table B3). Peripheral blood samples from the 13-week study animals were examined for presence of micronucleated polychromatic and normochromatic erythrocytes; no induction of micronuclei was observed in any of the dose groups (Appendix B, Table B4).

DISCUSSION

Organoperoxides are a relatively large class of chemicals to which a segment of the population is exposed in the work place. They are difficult to study because of their inherent instability. Few studies have been designed to characterize their toxicity, and such knowledge is limited largely to anecdotal reports of human exposure and cursory studies of acute toxicity. These reports have confirmed that organoperoxides induce chemical burns at the point of contact when administered in neat or concentrated form (Radding, 1977), but little is known regarding toxicity induced by repeated exposure. The present study was designed to examine the fate and toxicity of a representative of this important chemical class. t-BP was selected for the study because it is one of the more commonly used organoperoxides, is sufficiently stable to permit dose preparation and administration, and is sufficiently lipid-soluble to cross cell membranes and reach potential subcellular target sites.

Preliminary stability studies of t-BP confirmed that the compound is sufficiently stable to permit dose preparation and administration. However, its relatively rapid degradation in the presence of glutathione, one of numerous reducing agents found in biological systems, indicates that it should have a short half-life in intact animals. This assumption was confirmed by *in vitro* studies that demonstrated that relatively high concentrations of t-BP had half-lives of 4.0 and 10.4 minutes in human and rat blood, respectively. Enzymatic or chemical degradation in the presence of liver fractions was even more rapid, demonstrating that any t-BP absorbed into the body would be expected to have a very short half-life. Benzoic acid and t-butanol constituted at least 93% of the products of enzymatic and/or chemical degradation in *in vitro* systems. The stability of t-BP in a suspension of stomach contents was concentration dependent but was thought to be sufficient to permit some absorption of the parent molecule into stomach tissue.

t-BP was relatively stable on isolated skin; t-BP-derived radioactivity was absorbed into or bound to skin with continued contact (Table 2). Dermal absorption of t-BP-derived radioactivity was confirmed by the observation that when placed on the skin of living animals, approximately 16% of the radiolabel from a wide range of t-BP doses was absorbed and excreted in urine in 24 hours. Due to the rapid chemical and/or enzymatic degradation of t-BP in biological systems, it was not possible to determine if the radiolabel absorbed from skin represented parent compound or products of t-BP degradation. In any case, only traces of t-BP-derived radioactivity appeared to be retained in tissues following dermal or i.v. administration.

t-BP-derived material excreted in urine was not identified because preliminary studies showed a quantitative yield of t-butanol and benzoic acid on degradation and/or metabolism. Because the radiolabel was in the benzoic acid portion of the molecule, it was assumed that material excreted in urine represented metabolites of benzoic acid. Results of previous studies conducted in this laboratory indicated that both rats and mice metabolize more than 90 percent of the benzoic acid (derived from benzyl acetate) to hippuric acid, and excrete it in urine (Abdo *et al.*, 1985). Further, in the previous study, metabolism and elimination of benzoic acid was observed to be linear with dose, with no evidence of saturation at doses up to an equivalent of approximately 380 mg/kg benzoic acid in the rat and over 700 mg/kg in the mouse.

Corn oil was used as a vehicle for gavage administration of t-BP and for molar equivalent doses of benzoic acid and t-butanol in the 14-day studies. t-BP and t-butanol were soluble in corn oil; benzoic acid was administered as a suspension. Signs of toxicity observed in rats were largely limited to increased stomach weights in males receiving the highest dose of t-BP. Food consumption and weight gain were little affected in mice receiving any dose of t-BP or t-butanol; there was little effect of t-butanol administration on any parameter measured. Significant organ-weight effects observed in rats and mice receiving t-BP were limited to increased stomach weights in both sexes receiving the higher doses. Histologic lesions induced by t-BP were limited to the forestomach and were characterized by hyperplasia, ulceration, and acute inflammation.

Mice appeared to be more sensitive to toxic effects of benzoic acid than rats; both sexes showed symptoms of intoxication. Toxicity appeared to be dose-dependent, and 3 of 5 male mice and 1 of 5 female mice in the highest-dose groups (642 mg/kg) did not survive the 14-day treatment. Weight gain in female mice receiving benzoic acid was significantly depressed in a dose-dependent fashion. Benzoic acid may have been more toxic than t-BP because it was administered as a suspension in corn oil. In this form, it may have rapidly partitioned from the oil into the stomach to result in a bolus dose. Animals that died following benzoic acid administration did not have notable lesions; they may have died from acidosis resulting from the rapid absorption of this acid, but this was not confirmed. On the other hand, since t-BP is quite soluble in corn oil, it would be expected to partition from the oil into the gastrointestinal tract more slowly as the oil was digested in the small intestine.

In the 13-week studies, t-BP was administered as a suspension in water, to more closely simulate possible human exposure which might result from ingestion. Any material ingested would be rapidly absorbed through the stomach rather than slowly absorbed from the intestines as with gavage in corn oil. Results of these studies indicate that toxicity resulting from t-BP administration was minimal in both rats and mice. In rats, there was a slight depression in food consumption; body weight gains of both sexes in the highest dose group were significantly depressed after week 7. No clinical effects were observed which could be attributed to t-BP administration. Variations in organ weights were largely restricted to increased stomach weights in both male and female rats. Both the glandular stomach and forestomachs were affected, but the effect on the forestomach was much greater (Table 8). Dose-dependent forestomach hyperplasia was observed in all dosed male rats and in all females except those receiving the lowest dose.

Mice receiving t-BP for 13 weeks exhibited few symptoms of intoxication. Survival was good, and no deaths were attributed to t-BP administration. Neither food consumption nor body-weight gains were affected significantly; no clinical toxicity or gross lesions observed at necropsy could be attributed to chemical administration. Evidence of t-BP toxicity was limited to increased forestomach weight and dose-related hyperplasia in both sexes (Table 10). Glandular stomach weights were increased slightly, but no histopathological lesions were observed.

In summary, results of this study indicate that oral gavage administration of t-BP at doses up to 500 mg/kg produced little or no toxicity past the point of initial contact, the stomach. Toxicity observed in the stomach, primarily the forestomach, was due probably to the inherent reactivity of t-BP to release free radicals which in turn reacted with the cell membranes of the stomach mucosa. However, the reactivity of t-BP also accounts for its very short half-life in biological systems. Its

reaction with stomach contents, stomach tissue, and blood probably prevented t-BP from reaching the systemic circulation and thus accounted for its lack of systemic toxicity. The degradation products of t-BP, which are t-butanol and benzoic acid, are likely to be absorbed into the systemic circulation, but they are relatively nontoxic. Both t-butanol and benzoic acid have been the subjects of other studies conducted by the National Toxicology Program and other organizations; they do not appear to be carcinogens or to produce other chronic toxicity.

Based on the observations described in this report, it is concluded that toxicity resulting from human exposure to t-BP would most likely be limited to the site of contact. On contact with human tissues, t-BP would be expected to cause local irritation of the skin and nasobronchial epithelium such as has been described in the literature (Radding, 1977). It is not likely that t-BP would be ingested in sufficient quantities to cause appreciable gastric toxicity; if ingested, t-BP would not be expected to gain access to the systemic circulation of humans to result in toxicity such as has been described for free radicals generated intracellularly (Kehrer, 1988).

REFERENCES

Abdo, K.M., Huff, J.E., Haseman, J.K., Boorman, G.A., Eustis, S.L., Matthews, H.B., Burka, L.T., Prejean, J.D., and Thompson, R.B. (1985) Benzyl acetate carcinogenicity, metabolism, and disposition in Fisher 344 rats and B6C3F$_1$ mice. *Toxicology* **37**, 159-170.

Bock, F.G., Myers, H.K., and Fox, H.W. (1975) Carcinogenic activity of peroxy compounds. *J. Nat. Can. Inst.* **55**, 1359-1361.

Boorman, G.A., Hickman, R.L., Davis, G.W., Rhode, L.S., White, N.W., Griffin, T.A., Mayo, J., and Hamm, T.E., Jr. (1986) Serological titers to murine viruses in 90-day and 2-year studies, in T.E. Hamm, Jr. (ed.), *Complications of Viral and Mycoplasmal Infections in Rodents to Toxicology Research and Testing*. New York: Hemisphere, pp. 11-23.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J. (1985) Quality assurance in pathology for rodent carcinogenicity studies, in H. Milman and E. Weisburger (eds.), *Handbook of Carcinogen Testing*. Park Ridge, NH: Noyes Publications, pp. 345-357.

Dixon, W., and Massey, F. (1951) *Introduction to Statistical Analysis*. New York: McGraw-Hill, pp. 145-147.

Dunn, O.J. (1964) Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Emergency Response Guidebook (1987) DOT Publication 5800.4. Washington, D.C.: U.S. Government Printing Office, G-48.

Free-radical initiators (1983-1984), in *Modern Plastics Encyclopedia*, Vol. 60, #12A. New York: McGraw-Hill, Inc., p. 624.

Galloway, S., Armstrong, M., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B., Resnick, M., Anderson, B., and Zeiger, E. (1987) Chromosome aberration and sister-chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Molec. Mutagen.* **10** (Suppl 10), 1-176.

Galloway, S., Bloom, A., Resnick, M., Margolin, B., Nakamura, F., Archer, P., and Zeiger, E. (1985) Development of a standard protocol for *in vitro* cytogenetic testing with CHO cells: Comparison of results for 22 compounds in two laboratories. *Environ. Mutagen.* **7**, 1-52.

Hollander, M., and Wolfe, D. A. (1973) *Nonparametric Statistical Methods*. New York: John Wiley & Sons, pp. 120-123.

Kehrer, J.P., Mossman, B.T., Sevanian, A., Trush, M.A., and Smith, M.T. (1988) Contemporary issues in toxicology: Free-radical mechanisms in chemical pathogenesis. *Toxicol. Appl. Pharmacol.* **95**, 349-362.

MacGregor, J.T., Wehr, C.M., and Langlois, R.G. (1983) A simple fluorescent staining procedure for micronuclei and RNA in erythrocytes using Hoechst 33258 and pyronin Y. *Mutat. Res.* **120**, 269-275.

Maronpot, R.R., and Boorman, G.A. (1982) Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Mohan, V.K. (1982) Hazard evaluation of organic peroxides. *J. Hazard. Mater.* **5**, 197-220.

Morrison, D.F. (1976) *Multivariate Statistical Methods*. New York: McGraw Hill, Inc., pp. 170-179.

Morrissey, R.E., Schwetz, B.A., Lamb, J.C., IV, Ross, M.C., Teague, J.L., and Morris, R.W. (1988) Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program thirteen-week studies. *Fundam. Appl. Toxicol.* **11**, 343-358.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., and Zeiger, E. (1986) Salmonella mutagenicity tests. II. Results from the testing of 270 chemicals. *Environ. Mutagen.* **8** (Suppl 7), 1-119.

Plunkett, E.R. (1976) *Handbook of Industrial Toxicology.* New York: Chemical Publishing Company, Inc., pp. 308-309.

Radding, S.B. (1977) *Review of the Environmental Fate of Selected Chemicals*, EPA-560/5-77-003. Washington D.C.: U.S. Environmental Protection Agency, Office of Toxic Substances, pp. 63-67.

Rao, G.N., Haseman, J.K., and Edmondson, J. (1989) Influence of viral infections on body weight, survival, and tumor prevalence in Fischer 344/NCr rats on two-year studies. *Lab. Animal Sci.* **39**, 389-393.

Rao, G.N., Piegorsch, W.W., Crawford, D.D., Edmondson, J., and Haseman, J.K. (1989) Influence of viral infections on body weight, survival, and tumor prevalence of $B6C3F_1$ (C57BL/6N X C3H/HeN) Mice in Carcinogenicity Studies. *Fundam. Appl. Toxicol.* **13**, 156-164.

Sharratt, M., Frazer, A.C., and Forbes, O.C. (1964) Study of the biological effects of benzoyl peroxide. *Fd. Cosmet. Toxicol.* **2**, 527-538.

Shirley, E. (1977) A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

U.S. International Trade Commission (USITC) (1988) *Synthetic Organic Chemicals, U.S. Production and Sales.* Washington, D.C.: U.S. Government Printing Office, USITC Publication 2219.

Van Duuren, B.L. (1963) Carcinogenicity of epoxides, lactones, and peroxy compounds. *J. Nat. Can. Inst.* **31**(1), 41-55.

Williams, D.A. (1986) A note on Shirley's nonparametric test for comparing several dose levels with a zero control. *Biometrics* **42**, 183-186.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1988) Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Molec. Mutagen.* **11** (Suppl. 12), 1-158.

APPENDIX A

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

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Gavage Studies of *t*-Butyl Perbenzoate[1]

	Vehicle Control	30 mg/kg	60 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
MALE						
n	10	10	10	10	10	10
Necropsy body wt (g)	362 ± 8	376 ± 12	366 ± 6	361 ± 5	361 ± 5	338 ± 5*
Brain						
Absolute	1 97 ± 0 01	1 97 ± 0 02	1 98 ± 0 01	1 99 ± 0 02	1 99 ± 0 03	1 94 ± 0 02
Relative	5 45 ± 0 10	5 28 ± 0 14	5 42 ± 0 09	5 51 ± 0 06	5 52 ± 0 08	5 77 ± 0 09*
Heart						
Absolute	1 09 ± 0 03	1 09 ± 0 04	1 09 ± 0 02	1 05 ± 0 02	1 07 ± 0 02	1 00 ± 0 02*
Relative	3 00 ± 0 05	2 89 ± 0 04	2 98 ± 0 04	2 91 ± 0 05	2 97 ± 0 05	2 95 ± 0 05
Right Kidney						
Absolute	1 35 ± 0 03	1 36 ± 0 04	1 38 ± 0 03	1 40 ± 0 02	1 36 ± 0 03	1 29 ± 0 02
Relative	3 72 ± 0 05	3 63 ± 0 03	3 77 ± 0 07	3 89 ± 0 05	3 78 ± 0 08	3 82 ± 0 08
Liver						
Absolute	14 80 ± 0 35	15 35 ± 0 81	15 76 ± 0 46	14 92 ± 0 42	15 41 ± 0 56	13 84 ± 0 32
Relative	4 09 ± 0 09	4 06 ± 0 09	4 30 ± 0 10	4 13 ± 0 09	4 27 ± 0 14	4 10 ± 0 09
Lung						
Absolute	1 84 ± 0 06	1 87 ± 0 06	1 84 ± 0 06	1 83 ± 0 05	1 84 ± 0 04	1 75 ± 0 07
Relative	5 09 ± 0 13	5 00 ± 0 15	5 04 ± 0 15	5 06 ± 0 16	5 11 ± 0 09	5 17 ± 0 18
Spleen						
Absolute	0 82 ± 0 02	0 82 ± 0 03	0 82 ± 0 03	0 80 ± 0 02	0 79 ± 0 01	0 66 ± 0 01**
Relative	2 26 ± 0 04	2 17 ± 0 05	2 23 ± 0 07	2 22 ± 0 04	2 18 ± 0 02	1 94 ± 0 04**
Right Testis						
Absolute	1 43 ± 0 02	1 47 ± 0 03	1 46 ± 0 03	1 45 ± 0 01	1 48 ± 0 03	1 46 ± 0 02
Relative	3 95 ± 0 06	3 94 ± 0 07	3 99 ± 0 08	4 02 ± 0 06	4 11 ± 0 08	4 32 ± 0 07**
Thymus						
Absolute	0 37 ± 0 01	0 40 ± 0 02	0 40 ± 0 01	0 40 ± 0 02	0 36 ± 0 02	0 31 ± 0 013*
Relative	1 02 ± 0 03	1 07 ± 0 04	1 10 ± 0 04	1 10 ± 0 05	0 99 ± 0 04	0 93 ± 0 04
Forestomach						
Absolute	0 47 ± 0 02	0 46 ± 0 03	0 48 ± 0 02	0 59 ± 0 05	0 58 ± 0 03*	0 65 ± 0 02**
Relative	1 31 ± 0 08	1 22 ± 0 10	1 30 ± 0 04	1 64 ± 0 16	1 60 ± 0 08*	1 94 ± 0 08**
Glandular Stomach						
Absolute	1 41 ± 0 04	1 50 ± 0 11	1 39 ± 0 04	1 42 ± 0 07	1 41 ± 0 03	1 51 ± 0 07
Relative	3 89 ± 0 09	3 97 ± 0 19	3 79 ± 0 09	3 91 ± 0 15	3 90 ± 0 12	4 48 ± 0 18*
FEMALE						
n	9	10	10	10	9	10
Necropsy body wt (g)	201 ± 7	199 ± 4	195 ± 3	194 ± 3	200 ± 5	187 ± 4*
Brain						
Absolute	1 78 ± 0 02	1 82 ± 0 03	1 81 ± 0 02	1 80 ± 0 01	1 82 ± 0 02	1 79 ± 0 02
Relative	8 94 ± 0 29	9 16 ± 0 17	9 32 ± 0 10	9 28 ± 0 10	9 14 ± 0 23	9 61 ± 0 20*

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Gavage Studies of *t*-Butyl Perbenzoate (continued)

	Vehicle Control	30 mg/kg	60 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
FEMALE (continued)						
Heart						
Absolute	0 72 ± 0 02	0 71 ± 0 02	0 67 ± 0 02	0 68 ± 0 03	0 68 ± 0 01	0 68 ± 0 03
Relative	3 59 ± 0 12	3 55 ± 0 06	3 45 ± 0 07	3 51 ± 0 12	3 42 ± 0 08	3 63 ± 0 10
Right Kidney						
Absolute	0 74 ± 0 02	0 76 ± 0 02	0 76 ± 0 01	0 76 ± 0 01	0 83 ± 0 02**	0 75 ± 0 02
Relative	3 70 ± 0 05	3 82 ± 0 07	3 91 ± 0 05*	3 91 ± 0 07*	4 18 ± 0 07**	4 03 ± 0 10**
Liver						
Absolute	7 27 ± 0 30	7 05 ± 0 22	7 03 ± 0 17	6 98 ± 0 22	7 53 ± 0 14	6 53 ± 0 27
Relative	3 62 ± 0 08	3 56 ± 0 12	3 62 ± 0 09	3 60 ± 0 09	3 78 ± 0 06	3 50 ± 0 12
Lung						
Absolute	1 23 ± 0 05	1 22 ± 0 04	1 27 ± 0 03	1 24 ± 0 04	1 24 ± 0 04	1 22 ± 0 04
Relative	6 14 ± 0 18	6 16 ± 0 10	6 54 ± 0 14	6 36 ± 0 16	6 24 ± 0 21	6 50 ± 0 12
Spleen						
Absolute	0 51 ± 0 01	0 50 ± 0 02	0 48 ± 0 01	0 49 ± 0 01	0 50 ± 0 01	0 45 ± 0 01**
Relative	2 53 ± 0 06	2 51 ± 0 08	2 44 ± 0 05	2 52 ± 0 06	2 50 ± 0 06	2 42 ± 0 04
Thymus						
Absolute	0 29 ± 0 02	0 28 ± 0 08	0 28 ± 0 01	0 26 ± 0 01	0 27 ± 0 01	0 26 ± 0 01
Relative	1 42 ± 0 04	1 44 ± 0 05	1 42 ± 0 05	1 34 ± 0 07	1 38 ± 0 05	1 39 ± 0 04
Forestomach						
Absolute	0 30 ± 0 01	0 35 ± 0 04	0 37 ± 0 02*	0 34 ± 0 02*	0 43 ± 0 03**	0 47 ± 0 03**
Relative	1 51 ± 0 10	1 74 ± 0 17	1 91 ± 0 11*	1 75 ± 0 08	2 14 ± 0 14**	2 52 ± 0 15**
Glandular Stomach						
Absolute	0 98 ± 0 03	1 0 ± 0 04	0 94 ± 0 02	0 95 ± 0 03	1 1 ± 0 03**	1 1 ± 0 04**
Relative	4 91 ± 0 19	5 17 ± 0 25	4 86 ± 0 12	4 92 ± 0 10	5 64 ± 0 15**	6 05 ± 0 25**

[1] Organ weights are given in grams, organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

* Statistically significantly different (P≤0 05) from the control group by Dunn's test or Shirley's test

** Statistically significantly different (P≤0 01) from the control group by Dunn's test or Shirley's test

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Studies of *t*-Butyl Perbenzoate[1]

	Vehicle Control	30 mg/kg	60 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
MALE						
n	9	10	10	10	10	10
Necropsy body wt (g)	34 7 ± 1 1	35 9 ± 1 4	35 5 ± 0 6	35 17 ± 0 9	34 9 ± 1 3	34 6 ± 1 3
Brain						
Absolute	0 443 ± 0 006	0 445 ± 0 008	0 448 ± 0 005	0 444 ± 0 006	0 449 ± 0 006	0 446 ± 0 006
Relative	12 8 ± 0 4	12 5 ± 0 4	12 7 ± 0 3	12 7 ± 0 3	13 0 ± 0 4	13 0 ± 0 4
Heart						
Absolute	0 156 ± 0 004	0 169 ± 0 008	0 175 ± 0 010	0 171 ± 0 007	0 156 ± 0 006	0 163 ± 0 005
Relative	4 50 ± 0 10	4 74 ± 0 24	4 94 ± 0 26	4 87 ± 0 19	4 51 ± 0 21	4 79 ± 0 27
Right Kidney						
Absolute	0 266 ± 0 007	0 298 ± 0 011	0 285 ± 0 007	0 280 ± 0 006	0 289 ± 0 010	0 281 ± 0 008
Relative	7 68 ± 0 19	8 29 ± 0 14	8 03 ± 0 14	7 98 ± 0 08	8 33 ± 0 24	8 15 ± 0 18
Liver						
Absolute	1 54 ± 0 05	1 60 ± 0 06	1 63 ± 0 05	1 61 ± 0 03	1 70 ± 0 07	1 63 ± 0 07
Relative	44 6 ± 1 2	44 9 ± 1 4	46 0 ± 1 0	46 0 ± 1 1	48 7 ± 1 0*	47 2 ± 1 3
Lung						
Absolute	0 361 ± 0 012	0 326 ± 0 016	0 357 ± 0 012	0 345 ± 0 020	0 346 ± 0 012	0 331 ± 0 011
Relative	10 4 ± 0 3	9 1 ± 0 3	10 1 ± 0 3	9 8 ± 0 5	10 0 ± 0 3	9 6 ± 0 3
Spleen						
Absolute	0 070 ± 0 001	0 074 ± 0 001	0 070 ± 0 003	0 069 ± 0 002	0 070 ± 0 002	0 085 ± 0 017
Relative	2 02 ± 0 05	2 08 ± 0 07	1 98 ± 0 08	1 97 ± 0 06	2 00 ± 0 06	2 44 ± 0 44
Right Testis						
Absolute	0 117 ± 0 003	0 116 ± 0 002	0 119 ± 0 003	0 115 ± 0 003	0 118 ± 0 003	0 118 ± 0 004
Relative	3 38 ± 0 07	3 27 ± 0 10	3 35 ± 0 08	3 27 ± 0 08	3 41 ± 0 11	3 44 ± 0 10
Thymus						
Absolute	0 051 ± 0 003	0 053 ± 0 002	0 049 ± 0 002	0 049 ± 0 003	0 048 ± 0 004	0 054 ± 0 005
Relative	1 47 ± 0 05	1 48 ± 0 06	1 38 ± 0 05	1 39 ± 0 08	1 38 ± 0 11	1 55 ± 0 10
Forestomach						
Absolute	0 100 ± 0 009	0 084 ± 0 010	0 119 ± 0 015	0 114 ± 0 005	0 158 ± 0 010**	0 157 ± 0 004**
Relative	2 89 ± 0 28	2 38 ± 0 30	3 39 ± 0 47	3 24 ± 0 13	4 58 ± 0 33**	4 58 ± 0 15**
Glandular Stomach						
Absolute	0 196 ± 0 011	0 183 ± 0 005	0 199 ± 0 011	0 185 ± 0 006	0 198 ± 0 010	0 204 ± 0 006
Relative	5 66 ± 0 32	5 10 ± 0 08	5 64 ± 0 32	5 28 ± 0 16	5 74 ± 0 35	5 97 ± 0 25
FEMALE						
n	10	10	10	10	9	10
Necropsy body wt (g)	30 4 ± 1 1	29 4 ± 0 8	30 4 ± 1 1	30 6 ± 1 2	27 9 ± 0 8	30 6 ± 1 2
Brain						
Absolute	0 466 ± 0 005	0 467 ± 0 007	0 471 ± 0 007	0 478 ± 0 005	0 464 ± 0 009	0 470 ± 0 006
Relative	15 5 ± 0 5	16 0 ± 0 5	15 6 ± 0 6	15 8 ± 0 6	16 7 ± 0 4	15 6 ± 0 6

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Studies of *t*-Butyl Perbenzoate (continued)

	Vehicle Control	30 mg/kg	60 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
FEMALE (continued)						
Heart						
Absolute	0 134 ± 0 005	0 139 ± 0 005	0 149 ± 0 007	0 152 ± 0 010	0 146 ± 0 009	0 142 ± 0 004
Relative	4 47 ± 0 24	4 72 ± 0 16	4 93 ± 0 26	4 95 ± 0 21	5 24 ± 0 26	4 68 ± 0 16
Right Kidney						
Absolute	0 202 ± 0 007	0 210 ± 0 004	0 206 ± 0 006	0 211 ± 0 005	0 205 ± 0 005	0 214 ± 0 006
Relative	6 65 ± 0 13	7 19 ± 0 28	6 83 ± 0 24	6 96 ± 0 25	7 36 ± 0 18**	7 05 ± 0 18*
Liver						
Absolute	1 22 ± 0 04	1 20 ± 0 03	1 29 ± 0 05	1 27 ± 0 03	1 18 ± 0 03	1 26 ± 0 04
Relative	40 2 ± 0 8	40 9 ± 1 3	42 4 ± 1 2	41 7 ± 1 2	42 1 ± 0 6	41 6 ± 1 1
Lung						
Absolute	0 325 ± 0 017	0 323 ± 0 013	0 303 ± 0 015	0 335 ± 0 014	0 315 ± 0 024	0 297 ± 0 018
Relative	10 9 ± 0 7	11 0 ± 0 4	10 0 ± 0 6	11 0 ± 0 4	11 2 ± 0 8	9 8 ± 0 5
Spleen						
Absolute	0 084 ± 0 001	0 085 ± 0 002	0 087 ± 0 002	0 087 ± 0 003	0 085 ± 0 003	0 100 ± 0 006**
Relative	2 80 ± 0 10	2 89 ± 0 09	2 89 ± 0 10	2 86 ± 0 13	3 06 ± 0 08	3 29 ± 0 22
Thymus						
Absolute	0 061 ± 0 004	0 064 ± 0 004	0 062 ± 0 003	0 061 ± 0 002	0 060 ± 0 004	0 066 ± 0 003
Relative	2 00 ± 0 11	2 16 ± 0 10	2 03 ± 0 08	1 99 ± 0 09	2 16 ± 0 11	2 18 ± 0 10
Forestomach						
Absolute	0 098 ± 0 014	0 117 ± 0 017	0 110 ± 0 008	0 121 ± 0 006	0 145 ± 0 006**	0 184 ± 0 007**
Relative	3 25 ± 0 45	3 97 ± 0 57	3 65 ± 0 28	4 00 ± 0 24	5 26 ± 0 30**	6 10 ± 0 32**
Glandular Stomach						
Absolute	0 207 ± 0 005	0 196 ± 0 006	0 189 ± 0 007	0 207 ± 0 011	0 221 ± 0 011	0 238 ± 0 006*
Relative	6 86 ± 0 18	6 68 ± 0 22	6 27 ± 0 27	6 82 ± 0 35	7 96 ± 0 41	7 83 ± 0 24

[1] Organ weights are given in grams, organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

* Statistically significantly different (P≤0 05) from the control group by Dunn s test or Shirley's test

** Statistically significantly different (P≤0 01) from the control group by Dunn's test or Shirley's test

APPENDIX B
Genetic Toxicology

Table B1 Mutagenicity of t-Butyl Perbenzoate in *Salmonella typhimurium* [a]

DOSE	REVERTANTS/PLATE[b]								
mg/Plate	(-) S9			10% Hamster S9			10% Rat S9		
TA100									
0 0	105 ± 6 7	88 ± 1 2	91 ± 5 9	166 ± 4 5	182 ± 6 7	100 ± 12 4	142± 2 8	145± 10 2	141 ± 3 5
0 3		76 ± 1 5	85 ± 4 3						
1 0	96 ± 5 9	79 ± 0 7	104 ± 1 2						
3 3	93 ± 5 4	89 ± 8 2	118 ± 0 9	153 ± 5 7			129 ± 2 3		
10 0	92 ± 2 5	110 ± 3 8	129 ± 7 5	190 ± 5 0	147 ± 7 9	147 ± 4 6	159 ± 10 7	145 ± 7 2	161 ± 7 2
16 0					178 ± 7 1	127 ± 7 3		147 ± 5 5	165 ± 12 0
33 0	Toxic	126 ± 5 0	Toxic	287 ± 8 8	232 ± 18 7	146 ± 4 4	287 ± 19 0	213 ± 14 3	182 ± 4 7
66 7					268 ± 8 2	227 ± 14 0		266 ± 1 9	246 ± 24 6
100 0	Toxic			208 ± 18 5	136 ± 21 4	269 ± 4 4	149 ± 4 6	Toxic	288 ± 8 1
167 0									
333 0				Toxic			Toxic		
Trial summary	Negative	Weakly Positive	Weakly Positive	Weakly Positive	Weakly Positive	Positive	Equivocal	Weakly Positive	Positive
Positive control[c]	1486 ± 26 4	738 ± 23 6	1616 ± 45 9	2642 ± 45 8	2045 ± 109 9	1248 ± 54 5	2645 ± 18 4	2393 ± 65.2	1060 ± 71.2
TA1535									
0 0	10 ± 1 7	4 ± 1 2		20 ± 1 2	15 ± 1 5		11 ± 1 7	11 ± 1 3	
1 0	13 ± 1 9	4 ± 0 3			17 ± 2 8				
3 3	11 ± 2 6	5 ± 0 6		20 ± 3 7	16 ± 2 0		8 ± 0 6	13 ± 3 2	
10 0	9 ± 1 5	4 ± 0 9		22 ± 2 4	14 ± 0 9		10 ± 1 3	14 ± 3 5	
33 0	9 ± 2 3	6 ± 0 9		19 ± 5 0	12 ± 1 0		17 ± 2 9	9 ± 0 7	
100 0	13 ± 3 2	6 ± 1 5		12 ± 2 0	12 ± 0 3		12 ± 3 3	11 ± 1 5	
333 0				Toxic			Toxic	Toxic	
Trial summary	Negative	Negative		Negative	Negative		Negative	Negative	
Positive control[c]	1152 ± 144 3	643 ± 43 4		229 ± 10 1	278 ± 10 4		200 ± 24 1	283 ± 27 7	
TA1537									
0 0	9 ± 1 2	9± 2 0	3± 0 0	16 ± 2 9	21 ± 0 6	13 ± 0 9	15 ± 0 0	23 ± 1 0	6 ± 0 9
1 0	10 ± 0 6		3± 0 9						
3 3	10 ± 1 5		3± 0 9	15 ± 2 6			18 ± 3 5		
10 0	9 ± 2 4		6± 1 0	13 ± 3 4		20 ± 3 1	15 ± 2 3	22 ± 0 3	7 ± 1 5
16 0		11± 2 3			25 ± 1 8	19 ± 0 0		24 ± 2 7	14 ± 1 5
33 0	12 ± 2 1	17± 0 9	10± 2 7	56 ± 0 9	36 ± 0 9	15 ± 2 6	65 ± 7 8	48 ± 13 7	41 ± 5 3
66 7		21± 1 0		65 ± 10 4	30 ± 0 6			59 ± 12 3	26 ± 6 6
100 0	23 ± 2 1	Toxic	16 ± 3 1	46 ± 10 2	47 ± 7 0	64 ± 5 2	14 ± 2 2	Toxic	Toxic
167 0		Toxic			Toxic				
333 0				Toxic			Toxic		
Trial summary	Equivocal	Weakly Positive	Weakly Positive	Equivocal	Positive	Positive	Equivocal	Positive	Positive
Positive control[c]	161 ± 11 8	223 ± 101 7	62 ± 11 6	521 ± 27 1	216 ± 33 1	233 ± 14 4	483 ± 1 5	380 ± 120 7	337 ± 12 8

Table B1 Mutagenicity of t-Butyl Perbenzoate in *Salmonella typhimurium*[a] (continued)

DOSE	REVERTANTS/PLATE[b]								
mg/Plate	(-) S9			10% Hamster S9			10% Rat S9		
TA98									
0 0	22 ± 2 3	11 ± 0 3	15± 2 6	37 ± 2 8	40 ± 0 6	29 ± 3 8	29 ± 1 5	44 ± 0 9	31 ± 1 5
1 0	20 ± 3 8	14 ± 0 6	17± 2 2						
3 3	27 ± 2 3	17 ± 2 0	18 ± 2 6	38 ± 1 5			32 ± 3 2		
10 0	28 ± 2 4	16 ± 0 9	21± 3 5	44 ± 3 4	44 ± 2 6	54 ± 5 0	43 ± 3 5	41 ± 2 9	53 ± 5 5
16 0					60 ± 2 6	41 ± 2 3	50 ± 2 1	56 ± 2 9	
33 0	20 ± 4 3	25 ± 4 1	26 ± 0 6	65 ± 6 8	76 ± 6 4	45 ± 6 4	72 ± 0 6	77 ± 5 7	57 ± 7 0
66 7					55 ± 7 6	71 ± 6 8		23 ± 3 8	87 ± 5 5
100 0	28 ± 2 0	34 ± 5 2	36± 4 0	35 ± 5 2	Toxic	75 ± 11 8	33 ± 2 0	Toxic	76 ± 4 8
333 0				Toxic			Toxic		
Tnal summary	Negative	Positive	Positive	Weakly Positive	Weakly Positive	Positive	Positive	Weakly Positive	Positive
Positive control[c]	195 ± 27 9	188 ± 11 6	171 ± 9 0	1611 ± 75 1	1305 ± 219 9	595 ± 49 4	1747 ± 26 4	2223 ± 214 3	508 ± 34 4

a Study performed at Case Western Reserve University The detailed protocol and these data are presented in Mortelmans *et al* (1986) Cells and study compound or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver High dose was limited by toxicity or solubility, but did not exceed 10 mg/plate, 0 mg/plate dose is the solvent control

b Revertants are presented as mean ± the standard error from 3 plates

c Positive control, 2-aminoanthracene was used on all strains in the presence of S9 In the absence of metabolic activation, 4-nitro-o-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537

Table B2 Induction of Sister-Chromatid Exchanges in Chinese Hamster Ovary Cells by t-Butyl Perbenzoate

Dose (μg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCE/ Chromosome	SCE/ Cell	Hrs in BrdU	% Increase Over Solvent[b]
-S9[c]							
Trial 1--Summary: Positive							
Dimethylsulfoxide							
	50	1049	45	0 43	9 1	26 5	
t-Butyl Perbenzoate							
0 16	50	1050	468	0 44	9 4	26 5	2 54
0 50	50	1052	423	0 40	8 5	26 5	-7 50
1 60	50	1049	618	0 58	12 4	26 5	35 53*
5 00	50	1048	1066	1 01	21 3	26 5	134 00*
16 00	0						
Mitomycin-C[e]							
0 001	50	1049	585	0 55	11 7	26 5	28 29*
0 010	10	209	473	2 26	47 3	26 5	420 64*
			PROBABILITY: 0.000000[d]				
Trial 2--Summary: Positive							
Dimethylsulfoxide							
	50	1049	473	0 45	9	27 5	
t-Butyl Perbenzoate							
1 0	50	1049	584	0 55	11 7	27 5	23 47*
2 5	50	1048	749	0 71	15 0	27 5	58 50*
5 0	50	1049	1618	1 54	32 4	27 5	242 08*
Mitomycin-C[e]							
0 0008	50	1043	648	0 62	13 0	27 5	37 79*
0 0050	10	210	329	1 56	32 9	27 5	247 45*
			PROBABILITY: 0.000000				
+S9[e]							
Trial 1--Summary: Negative							
Dimethylsulfoxide							
	50	1037	370	0 35	7 4	26 0	
t-Butyl Perbenzoate							
0 5	50	1045	368	0 35	7 4	26 0	-1 30
1 6	50	1045	378	0 36	7 6	26 0	1 38
5 0	50	1037	341	0 32	6 8	26 0	-7 84
16 0	50	1039	434	0 41	8 7	26 0	17 07
50 0	0				26 0		
Cyclophosphamide[e]							
0 3	50	1042	646	0 61	12 9	26 0	73 76*
0 6	50	1038	807	0 77	16 1	26 0	117 90*
			PROBABILITY: 0.047212				

Table B2 Induction of Sister-Chromatid Exchanges in Chinese Hamster Ovary Cells by t-Butyl Perbenzoate (continued)

Dose (μg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCE/ Chromosome	SCE/ Cell	Hrs in BrdU	% Increase Over Solvent[b]
			+S9[e]				
Trial 2--Summary: Negative							
Dimethylsulfoxide							
	50	1046	413	0 39	8 3	26 5	
t-Butyl Perbenzoate							
1 0	50	1051	400	0 38	8 0	26 5	-3 61
5 0	50	1050	429	0 40	8 6	26 5	3 48
10 0	50	1048	439	0 41	8 8	26 5	6 09
Cyclophosphamide[e]							
0 3	50	1048	549	0 52	11 0	26 5	32 68*
			PROBABILITY: 0.123192				

a Study performed at Environmental Health Research & Testing SCE = sister chromatid exchange, BrdU = bromodeoxyundine A detailed description of the SCE protocol is presented by Galloway *et al* (1985, 1987) Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethylsulfoxide) as described in (c) and (d) below, and cultured for sufficient time to reach second metaphase division Cells were then collected by mitotic shake-off, fixed, air-dried, and stained

b SCE's/chromosome of culture exposed to study chemical relative to those of culture exposed to solvent

c In the absence of S9, cells were incubated with study compound or solvent for 2 h at 37°C Then BrdU was added and incubation was continued for 24 h Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 h

d Trend test

e In the presence of S9, cells were incubated with study compound or solvent for 2 h at 37°C The cells were then washed, and medium containing BrdU was added Cells were incubated for a further 26 h, with colcemid present for the final 2-3 h S9 was from the livers of Aroclor 1254-induced male Sprague Dawley rats

* Positive response

Table B3 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by t-Butyl Perbenzoate[a]

-S9[b]					+S9[c]				
Dose (µg/ml)	Total Cells	No. of Abs	Abs/Cell	% Cells w/ Abs	Dose (µg/ml)	Total Cells	No. of Abs	Abs/Cell	% Cells w/ Abs
Trial 1									
Harvest time: 12.5 hours					**Harvest time: 13.0 hours**				
Dimethylsulfoxide									
	100	1	0 01	1 0		100	1	0 01	1 0
t-Butyl perbenzoate									
5 0	100	9	0 09	9 0*	10 0	100	6	0 06	5 0
10 0	100	42	0 42	31 0*	16 0	100	9	0 09	9 0*
					30 0	100	15	0 15	14 0*
					50 0	100	22	0 22	20 0*
					100 0	34	26	0 76	26 0*
Summary: Positive					**Summary: Positive**				
Mitomycin-C[d]					**Cyclophosphamide[d]**				
25	100	33	0 33	27 0	15 0	100	45	0 4	38 0*
00	50	32	0 64	50 0					
		-S9[b]							
Trial 2									
Harvest time: 17.5 hours[d]									
Dimethylsulfoxide									
	100	3	0 03	3 0					
t-Butyl perbenzoate									
5 0	100	26	0 26	23 0*					
10 0	100	38	0 38	32 0*					
Summary: Positive									
Mitomycin-C[d]									
0 125	100	26	0 26	21 0*					
0 250	50	19	0 38	22 0*					

(a) Study performed at Environmental Health Research & Testing Abs = aberrations A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al* (1985, 1987) Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethylsulfoxide) as indicated in (b) and (d) Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake off, fixed, and stained in 6% Giemsa

(b) In the absence of S9, cells were incubated with study compound or solvent for 8-10 h at 37° C Cells were then washed and fresh medium containing colcemid was added for an additional 2-3 h followed by harvest.

(c) In the presence of S9, cells were incubated with study compound or solvent for 2 h at 37°C Cells were then washed, medium was added, and incubation was continued for 8-10 h Colcemid was added for the last 2-3 h of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague Dawley rats

(d) Because of significant chemical-induced cell cycle delay, incubation time prior to addition of colcemid was lengthened to provide sufficient metaphases at harvest

* Positive response

Table B4 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Exposed to t-Butyl Perbenzoate for 13 Weeks[a]

	MICRONUCLEATED CELLS/1000 CELLS[b]		
DOSE (mg/kg)	PCE	NCE	NUMBER OF MICE
Male mice			
0	2 53 ± 0 53	1 63 ± 0 10	9
30	2 20 ± 0 36	1 46 ± 0 09	10
60	2 20 ± 0 47	1 39 ± 0 12	10
125	2 17 ± 0 49	1 54 ± 0 15	10
250	1 76 ± 0 37	1 78 ± 0 12	10
500	48 ± 0 37	1 75 ± 0 19	10
Trend test[c]	**P = 0.9653**	**P = 0.2297**	
Female mice			
0	2 12 ± 0 45	1 12 ± 0 14	10
30	1 91 ± 0 35	1 22 ± 0 14	10
60	1 51 ± 0 50	1 06 ± 0 15	10
125	0 83 ± 0 26	1 11 ± 0 09	10
250	0 93 ± 0 32	0 98 ± 0 06	9
500	1 15 ± 0 37	1 28 ± 0 1	10
Trend test[c]	**P = 0.9770**	**P = 0.4995**	

(a) Smears were prepared from peripheral blood samples obtained by cardiac puncture of dosed and control animals at the time of terminal kill Slides were stained with Hoechst 333258/pyronin Y (MacGregor *et al*, 1983) At least 10000 NCE and 2000 PCE from each animal were scored for micronuclei No significant elevation in the frequency of micronucleated erythrocytes was observed in either male or female mice chronically exposed to t-butyl perbenzoate

(b) Mean ± standard error of the mean

(c) Cochran-Armitage linear regression of proportions for PCE's or linear contrasts from Analysis of Variance for NCE's