National Toxicology Program
Toxicity Report Series
Number 53

NTP Technical Report
on Toxicity Studies of

t-Butyl Alcohol

(CAS No. 75-65-0)

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

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

Note to the Reader

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- the National Cancer Institute (NCI) of the National Institutes of Health;
- the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health;
- the National Center for Toxicological Research (NCTR) of the Food and Drug Administration; and
- the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control.

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The studies described in this toxicity study report were performed under the direction of NIEHS and were conducted in compliance with NTP laboratory health and safety requirements. These studies met or exceeded all applicable federal, state, and local health and safety regulations. Animal care and use were in accord and compliance with the Public Health Service Policy on Humane Care and Use of Animals.

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This NTP report on the toxicity studies of *t*-butyl alcohol is based primarily on 18-day and 13-week studies that took place from August 1986 through June 1987.

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PEER REVIEW

The draft report on the toxicity studies of *t*-butyl alcohol was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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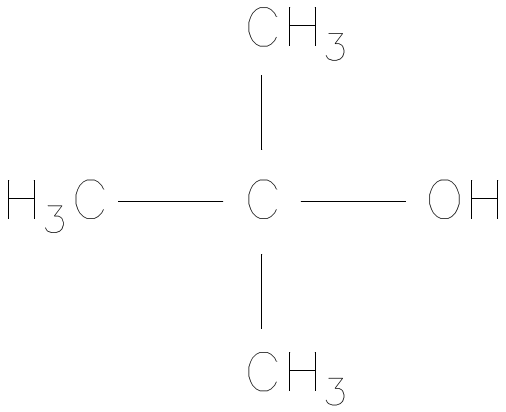
ABSTRACT



_t_-BUTYL ALCOHOL

CAS No. 75-65-0

Chemical Formula: $C_4H_{10}O$ Molecular Weight: 74.12

Synonyms: 2-Methyl-2-propanol, 2-methylpropan-2-ol, TBA, *t*-butanol, *t*-butyl hydroxide, trimethyl carbinol, trimethyl methanol

t-Butyl alcohol is widely used in the manufacture of perfumes and a variety of cosmetics. It is also used as a raw material in the production of isobutylene, which may be used to produce methyl tertiary butyl ether, a common gasoline additive, or to produce butyl elastomers used in the production of automobile tires. The National Cancer Institute nominated *t*-butyl alcohol to the NTP for study as a result of a review of chemicals found in drinking water. In addition to the high annual production and the potential for occupational exposure, there is also a potential for human exposure to *t*-butyl alcohol by the inhalation route from its use as an additive in unleaded gasoline. Therefore, toxicity studies of *t*-butyl alcohol were conducted in male and female F344/N rats and B6C3F_1 mice by whole-body inhalation. Animals were evaluated for hematology, clinical chemistry, urinalysis, reproductive toxicity, and histopathology. The genetic toxicity of *t*-butyl alcohol was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and L5178Y mouse lymphoma cells or sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, and by measuring the frequency of micronucleated erythrocytes in rat bone marrow and mouse peripheral blood.

In the 18-day inhalation studies, groups of five male and five female rats and mice were exposed to *t*-butyl alcohol by inhalation at concentrations of 450, 900, 1,750, 3,500, and 7,000 ppm for 6 hours per day, 5 days per week, for 12 exposure days. All rats and mice exposed to 7,000 ppm were killed moribund following a single 6-hour exposure. One 3,500 ppm male mouse died on day 3. Final mean body weights of 3,500 ppm male and female rats were significantly lower than those of the controls. Final mean body weights and body weight gains of all other exposed groups were similar to those of the controls. In animals exposed to 3,500 ppm, the thymus weights of male and female rats and female mice were less than those of the controls. The liver weights of male and female mice exposed to 3,500 ppm were greater than those of the controls. No gross or microscopic lesions were present in rats or mice.

In the 13-week inhalation studies, groups of 10 male and 10 female rats and mice were exposed to *t*-butyl alcohol at concentrations of 0, 135, 270, 540, 1,080, and 2,100 ppm for 6 hours per day, 5 days per week, for 13 weeks. One 2,100 ppm and five 1,080 ppm male mice died before the end of the studies. The final mean body weight of 2,100 ppm female mice and the mean body weight gains of 1,080 and 2,100 ppm female mice were significantly lower than those of the controls. Clinical findings of toxicity in the 1,080 ppm male mice that died during the studies included rough coats and emaciated appearance, hypoactivity, and prostration.

Minimal decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts occurred in the 1,080 and 2,100 ppm male rats at week 13. Hemoglobin concentrations and/or hematocrit values were also minimally decreased in male rats in the lower exposure groups. At week 13, a minimal decrease in urine pH occurred in the 1,080 ppm female and 2,100 ppm male and female rats. Neutrophilia occurred in the 2,100 ppm male mice. Organ weight differences in exposed rats included increased absolute and relative kidney weights of 1,080 ppm males and 2,100 ppm males and females and increased relative liver weights of 1,080 and 2,100 ppm females.

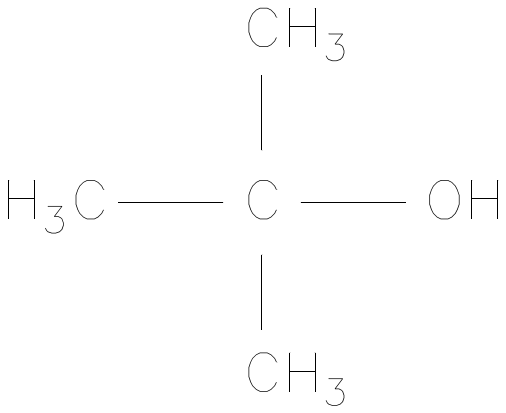
There were no treatment-related gross findings in male or female rats or mice; no microscopic lesions occurred in female rats or male or female mice that survived to the end of the study. In male rats, there was an exposure concentration-related increase in the severity of chronic nephropathy. Splenic lymphoid depletion was present in male mice that died during the studies; this lesion was presumed to be secondary to stress.

t-Butyl alcohol produced no adverse effects on reproductive parameters in male or female rats or mice.

The results of all tests of *t*-butyl alcohol for induction of genetic damage *in vitro* and *in vivo* were negative. *In vitro*, *t*-butyl alcohol was negative in *Salmonella typhimurium* and mouse lymphoma cell mutation tests, and it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells. These *in vitro* studies were conducted with and without metabolic activation (S9). *In vivo*, no increase in the frequency of micronucleated erythrocytes was observed in peripheral blood samples from mice administered *t*-butyl alcohol in drinking water for 13 weeks. Also, induction of micronucleated erythrocytes was noted in bone marrow cells of rats administered *t*-butyl alcohol by intraperitoneal injection.

In summary, inhalation exposure of rats and mice to *t*-butyl alcohol resulted in deaths following a single 7,000 ppm exposure and clinical findings of alcohol toxicity (hyper- and hypoactivity, ataxia) at concentrations of 900 ppm and greater in rats and 1,750 ppm and greater in mice. In 13-week studies at concentrations up to 2,100 ppm, only one death (that of a 2,100 ppm mouse) was attributed to chemical exposure. The most notable evidence of toxicity at the end of 13 weeks was limited to males and consisted of increased kidney weights, which correlated microscopically to increased severity of chronic nephropathy. Reproductive parameters in male and female rats and mice were unaffected after 13 weeks of exposure, and the results of all tests for genetic toxicity were negative.

INTRODUCTION



***t*-BUTYL ALCOHOL**

CAS No. 75-65-0

Chemical Formula: $C_4H_{10}O$ Molecular Weight: 74.12

Synonyms: 2-Methyl-2-propanol, 2-methylpropan-2-ol, TBA, *t*-butanol, *t*-butyl hydroxide, trimethyl carbinol, trimethyl methanol

PHYSICAL PROPERTIES, PRODUCTION, USE, AND HUMAN EXPOSURE

t-Butyl alcohol is a colorless, volatile liquid with a camphor-like odor; it forms rhombic crystals at temperatures below 25° C. It has a specific gravity of 0.783 and is soluble in water, alcohols, esters, ethers, and aromatic and aliphatic hydrocarbons (*Patty's Industrial Hygiene and Toxicology*, 1982; *Hawley's Condensed Chemical Dictionary*, 1987).

t-Butyl alcohol is used in perfumes and a variety of cosmetic products, in industrial cleaning compounds, in aerosol sprays to remove household dust, in the production of butyl elastomers for automobile tires, and as a raw material in the production of isobutylene, an intermediate in the production of the common gasoline additive, methyl tertiary butyl ether (Sexton *et al.*, 1986; *Hawley's Condensed Chemical Dictionary*, 1987; CIR Expert Panel, 1989). *t*-Butyl alcohol is an ethanol denaturant, a dehydrating agent, and a solvent. In 1979, the U.S. Environmental Protection Agency granted a waiver to allow the use of *t*-butyl alcohol as a gasoline octane booster (44 FR, 10530); however, this use is not widespread.

t-Butyl alcohol can be an indirect food additive when used in the preparation and application of coatings for paper and paperboard used in food containers (21 CFR, § 176.200) and as a surface lubricant for the manufacture of metallic articles that contact food (21 CFR, § 178.3910). Due to the variety of uses of *t*-butyl alcohol, potential routes of human exposure are topical, inhalation, and ingestion.

t-Butyl alcohol was identified in one of eight samples of mothers' milk obtained from women residing in four different urban areas (Pellizzari *et al.*, 1982). In addition, *t*-butyl alcohol has been found (but not quantified) in the drinking water of at least one city out of five surveyed for volatile organics (Coleman *et al.*, 1976) and in water samples taken before, during, and after treatment at a New Orleans area municipal water treatment facility (Dowty *et al.*, 1975). The latter report also identified *t*-butyl alcohol in water from a commercial deionizing-charcoal filtering unit.

United States production of *t*-butyl alcohol in 1991 was estimated at 2,990 billion pounds (*Chemical Economics Handbook*, 1993). Based on a survey conducted by the National Institute for Occupational Safety and Health (NIOSH) from 1981 to 1983, an estimated 171,419 workers are potentially exposed to *t*-butyl alcohol in the workplace (NIOSH, 1990). The Occupational Safety and Health Administration has established a permissible exposure limit of 100 ppm or 300 mg/m^3 for *t*-butyl alcohol (29 CFR, § 1910.1000).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

While no published literature was found quantitating the absorption of *t*-butyl alcohol, several studies suggest rapid absorption following oral exposure, inhalation exposure, or intraperitoneal injection. Compared with the primary and secondary alcohols, tertiary alcohols are much more stable biologically and are not readily metabolized. The possible routes of metabolism of tertiary alcohols are direct conjugation of the hydroxyl group with glucuronic acid and oxidation of one or more of the alkyl substituents (Williams, 1959). Early metabolism studies identified glucuronide conjugates of *t*-butyl alcohol in the urine of rabbits; approximately 24% of a single 4 mmol/kg gavage dose was excreted as the glucuronide (Kamil *et al.*, 1953).

t-Butyl alcohol is not readily metabolized; it cannot form an aldehyde or ketone by dehydrogenation and is not a substrate for alcohol dehydrogenase (Arslanian *et al.*, 1971; Videla *et al.*, 1982). However,

in vitro studies showed that *t*-butyl alcohol serves as a substrate for rat liver microsomal mixed function oxidase and is demethylated to yield small amounts of formaldehyde and acetone. The reaction pathway appears to involve the interaction of *t*-butyl alcohol with hydroxyl radicals generated by the microsomes from hydrogen peroxide (Cederbaum and Cohen, 1980; Cederbaum *et al.*, 1983). Baker *et al.* (1982) also found small, highly variable amounts of acetone in the urine and expired air of rats following intraperitoneal injection of 0.75 to 2 g/kg *t*-butyl alcohol, indicating a minimal amount of oxidative metabolism. A separate experiment by these investigators using labeled compound suggested that *t*-butyl alcohol was not the sole source of acetone, but may have stimulated production from other sources. Significant increases in blood concentrations of acetone have been reported following 1 to 2 g/kg intraperitoneal doses of *t*-butyl alcohol in rats (Yojay *et al.*, 1982).

In male Sprague-Dawley rats administered *t*-butyl alcohol orally or by intraperitoneal injection, liver microsomal enzyme activity was increased threefold 18 hours after the last dose (quantity not specified) (Bechtel and Cornish, 1972). The oral route appeared more effective in eliciting this response, and liver weights were not significantly elevated.

After 5 days of inhalation exposure to 500 ppm *t*-butyl alcohol, male Sprague-Dawley rats had a 36% increase in microsomal cytochrome P_{450} in the kidneys; liver and lung P_{450} values were unaffected. After 3 days of inhalation exposure to 2,000 ppm, rats had significantly elevated hepatic P_{450} (28%); kidney P_{450} was not affected, and lung P_{450} was slightly decreased. These results may indicate that a longer duration of exposure is required before enzyme induction can be observed in the kidney, whereas exposure concentration may be more important for induction in the liver (Aarstad *et al.*, 1985).

Four male Wistar rats were given a single oral dose of 2.54 g/kg *t*-butyl alcohol. Six hours after administration, hepatic glutathione concentration was slightly lower and lipoperoxidation (indicated by diene conjugate formation) was slightly greater than those of the controls (Videla *et al.*, 1982). Thurman *et al.* (1980) did not detect oxidation of *t*-butyl alcohol in perfused rat liver, based on changes in oxygen uptake or alterations in the levels of reducing cofactors.

t-Butyl alcohol is eliminated slowly from the blood of rats. Following intraperitoneal injection of 2 g/kg in female Sprague-Dawley rats, *t*-butyl alcohol essentially was not metabolized, as indicated by the nearly constant blood concentrations over a 12-hour period (Thurman and Pathman, 1975). In further investigations (Thurman *et al.*, 1980), female Sprague-Dawley rats were pretreated with *t*-butyl alcohol in saline (5.7% w/v) by gavage every 8 hours for 1 or 2.5 days and then were given sufficient *t*-butyl alcohol

to elevate their blood concentrations to between 125 and 150 mg/100 mL. Eighteen hours were required to eliminate *t*-butyl alcohol completely from the blood of rats pretreated for 2.5 days, and 26 hours were required following pretreatment for 1 day. The rate of elimination of 1.2 g *t*-butyl alcohol/kg body weight was 0.7 mmol/kg per hour and was identical for both pretreated groups. *t*-Butyl alcohol was eliminated from the rat only 6% to 7% as fast as ethanol, which is consistent with the hypothesis that *t*-butyl alcohol is eliminated very slowly, possibly by glucuronidation. In Sprague-Dawley rats, the high serum concentration (9 mmol/L) of *t*-butyl alcohol 6 hours after inhalation of 2,000 ppm was regarded as a possible reflection of the limited metabolism of the compound (Aarstad *et al.*, 1985).

t-Butyl alcohol is also eliminated slowly from the blood of mice. Single intraperitoneal doses of 8.1 mmol/kg administered to nine male Swiss-Webster mice were eliminated from the blood in 8 to 9 hours (McComb and Goldstein, 1979). The same mice then inhaled *t*-butyl alcohol for 3 days at a concentration sufficient to maintain a mean blood level of 8 mmol/L, and *t*-butyl alcohol was not detected in the blood 3 hours after the mice were removed from the vapor chamber. A single intraperitoneal dose of 8.1 mmol *t*-butyl alcohol/kg body weight was then administered (to an unspecified number of mice) 4 hours after the end of the 3-day inhalation period; no *t*-butyl alcohol was detected in the blood 3 hours later. The increased rate of elimination of *t*-butyl alcohol in animals previously exposed may be due to an increased conjugation. These results in mice are in contrast to the findings reported by Thurman *et al.* (1980) following pretreatment in rats.

Humans

No information related to the absorption, distribution, metabolism, or excretion of *t*-butyl alcohol in humans has been reported in the literature.

TOXICITY

Experimental Animals

The oral LD_{50} of *t*-butyl alcohol has been reported as 3.5 g/kg in rats and mice (Schaffarzick and Brown, 1952; NIOSH, 1970) and 3.6 g/kg in rabbits (Munch, 1972); 441 mg/kg is the reported LD_{50} for mice by intraperitoneal injection (Maickel and McFadden, 1979). The primary acute effects in animals are signs of alcoholic intoxication and hepatotoxicity.

Tertiary alcohols are metabolized slowly and incompletely, so their toxic effects are especially persistent. Most of the tertiary alcohols are central nervous system depressants. The sedative-intoxicant and withdrawal effects of *t*-butyl alcohol are nearly identical to those of ethanol (LeBlanc and Kalant, 1975; Bellin and Edmonds, 1976; Snell and Harris, 1980). *t*-Butyl alcohol is four to five times more potent than ethanol in producing physical dependence in rats and mice (McComb and Goldstein, 1979; Wood and Laverty, 1979; Thurman *et al.*, 1980), and severe withdrawal reactions including anorexia, self-mutilation, and death have occurred (Grant and Samson, 1981). The higher potency of *t*-butyl alcohol in producing dependence is probably related to its slower clearance from the body.

A dose of 25 mmol *t*-butyl alcohol/kg body weight administered in water by gavage to female Wistar rats resulted in an accumulation of triacylglycerols in the liver, accompanied by an early increase in palmitate uptake into triacylglycerols and a delayed enhancement of free fatty acid concentrations in the blood (Beaugé *et al.*, 1981). There were no significant changes in hepatic or blood phospholipid concentrations at 2, 5, or 20 hours or in the 4-hour lactate/pyruvate ratio.

In a study of five butyl alcohols (method not given), *t*-butyl alcohol was found to have only a slight inhibitory effect on rat liver mitochondrial respiration and phosphorylation (Thore and Baltscheffsky, 1965). *t*-Butyl alcohol was intraperitoneally administered in a 25% aqueous solution to male Sprague-Dawley rats 18 hours prior to giving carbon tetrachloride or measuring glutathione concentrations. Serum glutamate-pyruvate transaminase concentrations were used to evaluate carbon tetrachloride induced hepatotoxicity.
t-Butyl alcohol was found to potentiate hepatotoxicity due to carbon tetrachloride by a mechanism that did not alter hepatic glutathione concentrations or cause a loss of body weight (Harris and Anders, 1980).

Thirteen-week studies of *t*-butyl alcohol in drinking water were conducted by the NTP in conjunction with 2-year carcinogenicity studies (Lindamood *et al.*, 1992; NTP, 1995). Male and female F344/N rats and B6C3F$_1$ mice were exposed to 0, 2.5, 5, 10, 20, or 40 mg/mL. Chemical effects in both species included mortality, lower final mean body weights, and transitional epithelial hyperplasia and inflammation of the urinary bladder at higher exposure levels. In exposed rats, increases in kidney weights were associated microscopically with nephropathy in males and females and tubular hyaline droplet accumulation and mineralization in males. Also in male rats, urinary bladder inflammation and hyperplasia associated with crystalluria and bladder calculi were treatment effects.

Humans

In some patients, *t*-butyl alcohol is a mild skin irritant. When the individual components of a sunscreen preparation were patch-tested on a patient with an allergic contact dermatitis, *t*-butyl alcohol was determined to be the cause (Edwards and Edwards, 1982). No other adverse effects of *t*-butyl alcohol in humans have been reported in the literature.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

In a comparative study in Swiss-Webster mice on the postnatal developmental effects of prenatal administration of *t*-butyl alcohol or ethanol, a liquid diet containing either 0.5%, 0.75%, or 1.0% *t*-butyl alcohol or 3.6% ethanol was fed to pregnant mice from gestation days 6 to 20. The *t*-butyl alcohol diet resulted in dose-related reductions in the number of litters, litter size, and pup birth weights and an increase in the number of stillborn pups. Testing of the pups indicated that *t*-butyl alcohol was approximately five times more potent than ethanol in producing a developmental delay in postnatal physiological and psychomotor performance (Daniel and Evans, 1982).

In another comparative study on the induction of microcephaly in neonatal Long-Evans rats, *t*-butyl alcohol or ethanol was administered in the milk formula by cannulation on postnatal days 4 through 7 (Grant and Samson, 1982). Mean daily doses of *t*-butyl alcohol were 0.60, 1.44, 2.16, or 2.69 g/kg body weight and were of equal anesthetic value to the ethanol doses, calculated by membrane-to-buffer partition coefficients. Following the 4-day alcohol exposure, all animals were given a plain milk formula by cannulation until day 18. Microcephaly was present to a similar degree in both alcohol-treated groups but not in the controls. It was suggested that the general impairment of brain growth could be due to the membrane solubilizing properties of both alcohols.

In vitro studies showed that ethanol reduced the fertilizing capacity of mouse spermatozoa at concentrations commonly observed after ethanol ingestion by man or experimental animals (100 to 400 mg/100 mL). At similar concentrations, *t*-butyl alcohol had no effect on fertilization (Anderson *et al.*, 1982).

Pregnant mice of the CBA/J and C57ABL/6J strains were given *t*-butyl alcohol (10.5 mmol/kg) or an equivalent volume of tap water by gavage every 12 hours from gestation day 6 through day 18 (Faulkner *et al.*, 1989). On day 18, there were significantly more resorptions per litter in the mice receiving *t*-butyl alcohol than in the controls, but there was no interstrain difference. Blood concentration

profiles in the C57ABL/6J mice showed that the treatment regimen produced *t*-butyl alcohol concentrations in blood equivalent to teratogenic ethanol treatment. However, body weights of the survivors were not affected, and no significant abnormalities occurred in either strain of animals receiving *t*-butyl alcohol.

In a teratology assessment of *t*-butyl alcohol, 1-butanol, and 2-butanol, pregnant Sprague-Dawley rats were exposed by inhalation to 0, 2,000, 3,500, or 5,000 ppm *t*-butyl alcohol for 7 hours per day on gestation days 1 through 19 (Nelson *et al.*, 1989). Dams were sacrificed on day 20, and fetuses were examined for skeletal abnormalities or visceral defects. No dose-related reduction in fetal weight occurred with any of the butanol isomers, and concentrations 50 times the current permissible exposure limit (100 ppm) did not produce teratogenic effects. In further investigations by Nelson *et al.* (1991), pregnant Sprague-Dawley rats were exposed to 2,000 or 4,000 ppm *t*-butyl alcohol by inhalation for 7 hours per day on gestation days 1 through 19; males were similarly exposed for 6 weeks and were mated to unexposed females. The higher concentration of *t*-butyl alcohol was maternally toxic, causing reduced feed intake and weight gain. However, the few behavioral or neurochemical effects noted in the offspring on tests conducted through 90 days of age were not considered biologically significant.

In castrated rats, *t*-butyl alcohol was found to be four times as potent as ethanol in decreasing plasma concentrations of luteinizing hormone, which regulates testosterone production (Chapin *et al.*, 1980).

Humans

No information on the reproductive and developmental toxicity of *t*-butyl alcohol in humans has been reported in the literature.

CARCINOGENICITY

Experimental Animals

Two-year studies of *t*-butyl alcohol in drinking water have been conducted in F344/N rats and B6C3F$_1$ mice by the NTP (1995). In rats, incidences of renal tubule hyperplasia and adenoma were increased in exposed males; in addition, the severity of nephropathy and the incidence and severity of renal transitional cell hyperplasia were also increased in exposed males. Incidences of thyroid gland follicular cell hyperplasia and adenoma were increased in exposed male and female mice.

Humans

No information on the carcinogenicity of *t*-butyl alcohol in humans was found in the literature.

GENETIC TOXICITY

The published genotoxicity data for *t*-butyl alcohol are from two studies, both of which are presented in Appendix D of this report. Results showed no induction of mutations by *t*-butyl alcohol in any of four strains of *Salmonella typhimurium* (Zeiger *et al.*, 1987) or in L5178Y mouse lymphoma cells (McGregor *et al.*, 1988). Both studies were performed with and without S9.

STUDY RATIONALE AND DESIGN

The National Cancer Institute nominated *t*-butyl alcohol for study as a result of a review of chemicals found in drinking water and because of its large annual production and potential for human exposure. Inhalation and drinking water were chosen as the routes of exposure for the toxicity and carcinogenicity evaluations because of concerns regarding possible exposure to *t*-butyl alcohol from gasoline fumes and groundwater contamination. Toxicology and carcinogenesis studies with the drinking water route have been reported (NTP, 1995). Endpoints evaluated in the inhalation studies included histopathology and clinical pathology in F344/N rats and B6C3F$_1$ mice. The effect of *t*-butyl alcohol on reproduction was assessed by evaluation of testicular and epididymal spermatozoal parameters and determination of the length of the estrous cycle in animals in the 13-week inhalation studies. In addition, the genetic toxicology of *t*-butyl alcohol was studied in *in vitro* assays for mutation induction in *Salmonella typhimurium* and L5178Y mouse lymphoma cells, for sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, for induction of micronuclei in bone marrow cells of rats administered *t*-butyl alcohol by intraperitoneal injection, and for increased frequency of micronuclei in peripheral erythrocytes of mice exposed to *t*-butyl alcohol during the 13-week drinking water studies.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF *t*-BUTYL ALCOHOL

t-Butyl alcohol (CAS Number 75-65-0) was obtained in three lots from FBC Chemical Corporation (Lancaster, NY). Lot UN-1120 was used throughout the 18-day studies and for part of the 13-week studies. Lots F020487 and 070981 were used for the remainder of the 13-week studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). All lots of the chemical, a clear colorless liquid, were identified as *t*-butyl alcohol by infrared, ultraviolet-visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with those expected for the structure, and the infrared and nuclear magnetic resonance spectra were consistent with the literature spectra of *t*-butyl alcohol (*Aldrich,* 1983; *Sadtler Standard Spectra*). Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for *t*-butyl alcohol. Karl Fischer water analyses indicated 0.015% ± 0.003% water for lot UN-1120, 0.032% ± 0.002% water for lot F020487,
and 0.24% ± 0.01% water for lot 070981. Gas chromatography of lots UN-1120 and F020487 using two systems indicated one major peak and one impurity with a peak area greater than or equal to 0.1% relative to the major peak. Gas chromatography of lot 070981 using two systems indicated two impurities with a combined peak area of 0.291% relative to the major peak. The overall purity of each lot was determined to be greater than 99%.

An accelerated stability study performed by the analytical chemistry laboratory using gas chromatography indicated that *t*-butyl alcohol was stable as the bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature in amber glass bottles with Teflon®-lined lids. During the 13-week studies, stability was monitored by gas chromatography; no degradation of the bulk chemical was detected.

VAPOR GENERATION SYSTEM

Liquid *t*-butyl alcohol was delivered by a positive displacement metering pump to a slightly heated Sonimist Ultrasonic Spray Nozzle nebulizer (Heat Systems-Ultrasonics, Inc., Farmingdale, NY). Liquid droplets 0.1 to 50 μm in diameter were discharged from the nebulizer into a plenum chamber and were evaporated in a stream of filtered, compressed air pumped through the chamber at 50 psig. All tubing, lines, and connectors were of stainless steel or Teflon® and were slightly heated to prevent solidification of the

t-butyl alcohol. The vapor was drawn into a common distribution manifold and either passed undiluted into the highest exposure chamber or was diluted with filtered room air by means of calibrated dilution flow meters to achieve the desired lower concentrations for the other chambers. The flow rate through the chamber was controlled by calibrated orifice flowmeters located in the exhaust line immediately downstream of the chamber. The following parameters were recorded three times daily: the pressure differential between each exposure chamber and the room, the chamber temperature and relative humidity, and the flow through each of the calibrated dilution flowmeters. The H-2000 exposure chambers (Hazelton Systems, Inc., Aberdeen, MD) had a 2 m^3 volume.

CONCENTRATION MONITORING

t-Butyl alcohol vapor concentrations were measured by a infrared analyzer with absorbance at two different wavelengths. A wavelength of 3,326 μm was used to monitor the three lowest exposure chambers in the 18-day studies and the two lowest in the 13-week studies. A wavelength of 8,598 μm was used to monitor the two highest exposure chambers in the 18-day studies and the three highest in the 13-week studies. Vapor concentration was measured hourly during exposure periods by means of a movable probe which sequentially sampled 12 points within each chamber to ensure vapor concentration homogeneity.

CHAMBER CHARACTERIZATION

Vapor Concentration

Vapor concentration in each chamber was demonstrated to be uniform and stable. Target concentrations and average concentration measurements recorded during the studies are summarized in Table 1. Target chamber concentrations during the 18-day studies were 0, 450, 900, 1,750, 3,500, and 7,000 ppm. A correction factor of 1.2 was applied to the recorded vapor concentrations for the 13-week studies due to a discrepancy in the method of use of the infrared analyzer noted during the course of the 13-week studies. Due to this correction, nominal chamber concentrations of *t*-butyl alcohol during the 13-week studies were 0, 135, 270, 540, 1,080, and 2,100 ppm.

Concentration Buildup and Decay

During the 18-day and 13-week studies, buildup and decay rates for chamber concentrations were monitored with the Miran-80 infrared analyzer. Values were collected at 60-second intervals and plotted by computer. The time following the start of exposure required for the *t*-butyl alcohol concentration to reach 90% of the final stable concentration level was identified as the T_{90} for chamber wash-in, and the time following the termination of vapor generation required for the aerosol concentration to decay to 10% of the

stable concentration level was identified as the T_{10} for chamber wash-out. The measured mean T_{90} wash-in value of 9 minutes was in agreement with the theoretical value of 9.2 minutes; a T_{90} of 10 minutes was used during the 18-day and 13-week studies.

TABLE 1
Summary of Chamber Concentrations in the 18-Day and 13-Week Inhalation Studies of *t*-Butyl Alcohol in F344/N Rats and B6C3F$_1$ Mice

Target Concentration (ppm)	n[a]	Average Concentration[b] (ppm)
Rats		
18-Day Study		
450	72	457 ± 15
900	72	910 ± 16
1,750	72	1,750 ± 23
3,500	72	3,523 ± 64
7,000	6[c]	6,989 ± 46
13-Week Study		
113	66	134 ± 5
225	66	272 ± 9
450	66	542 ± 15
900	66	1,080 ± 25
1,750	66	2,101 ± 44
Mice		
18-Day Study		
450	72	457 ± 15
900	72	910 ± 16
1,750	72	1,751 ± 23
3,500	72	3,524 ± 61
7,000	6[c]	7,024 ± 170
13-Week Study		
113	66	134 ± 5
225	66	272 ± 9
450	66	542 ± 15
900	66	1,080 ± 25
1,750	66	2,101 ± 44

[a] For the 18-day studies, n=total number of readings. For the 13-week studies, n=number of days for which mean concentrations were determined.
[b] Mean ± standard deviation
[c] All rats and mice died or were killed moribund after 1 day of exposure; therefore, no readings were taken during the remainder of the 18-day studies.

Stability Studies

In order to identify possible degradation products caused by vaporization, cold trap samples were collected from the highest and lowest concentration chambers during the last hour of a 6-hour exposure period. These samples were analyzed by gas chromatography-mass spectrometry. This analysis was done with and without animals in the chambers. No degradation products were detected at concentrations equal to or greater than 1% of the *t*-butyl alcohol concentrations during the 18-day and 13-week studies. In addition, each exposure chamber, the control chamber, and the room air were monitored with an aerosol monitor (APS-33, TSI, Minneapolis, MN) both prior to and during the studies to detect any particulate material that might arise during any part of the generation process. No significant particulate material was detected.

TOXICITY STUDY DESIGNS

Core Studies

F344/N rats and B6C3F$_1$ mice used in the 18-day and 13-week studies were obtained from Taconic Laboratory and Animal Services (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 10 days (rats) or 11 days (mice) for the 18-day studies and 13 days for the 13-week studies and were 6 or 7 weeks old when the studies began. Before the beginning of the studies, two male and two female rats and three male and two female mice from the 18-day studies and five male and five female rats and mice from the 13-week studies were randomly selected for parasite evaluation and gross observation for disease. Blood samples for determination of antibody titers to rodent viruses and bacteria were collected from three male and two female rats and mice at three time points during the 18-day studies and from five male and five female rats and mice at the end of the quarantine period for the 13-week studies. Blood samples were also collected from five male and five female control rats and mice at the end of the 13-week studies; the results indicated no positive antibody titers (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). Additional details concerning the study design are listed in Table 2.

In the 18-day studies, groups of five male and five female rats and mice were exposed to *t*-butyl alcohol by whole body inhalation to target concentrations of 0, 400, 900, 1,750, 3,500, and 7,000 ppm for 6 hours plus T_{90} per day, 5 days per week (excluding weekends and a holiday), for 12 exposure days in an 18-day period. Based on mortality and decreased body weights of animals exposed to target concentrations of 3,500 and 7,000 ppm, exposure levels selected for the 13-week studies were 0, 113, 225, 450, 900, and 1,750 ppm. Groups of 10 male and 10 female rats and mice were exposed for 6 hours plus T_{90} per day, 5 days per week (excluding weekends and a holiday), for 13 weeks, with at least 2 consecutive exposure

days before sacrifice. Actual concentrations of *t*-butyl alcohol generated during the 13-week study were 135, 270, 540,1,080, and 2,100 ppm.

For all studies, rats and mice were housed individually. City water (Columbus, OH) was available *ad libitum*. NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form was available *ad libitum* except during exposure periods and urine collection periods (rats). For the 18-day studies, clinical findings were recorded twice daily (before and after exposure), and animals were weighed 5 days before exposures began, on day 8, and at the end of the studies. For the 13-week studies, clinical findings were recorded weekly and the animals were weighed 6 days before exposures began, weekly thereafter, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Complete necropsies were performed on all animals. In the 18-day studies, the brain, heart, right kidney, liver, lung, right testis, and thymus were weighed. Organs and tissues were examined for gross lesions and were fixed and preserved in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin. In the 18-day studies, complete histopathologic examinations were performed on all rats and mice in the 0, 3,500, and 7,000 ppm groups, and gross lesions were examined in rats and mice from all exposure groups. In the 13-week studies, the brain, right epididymis, heart, right kidney, liver, lung, right testis, and thymus were weighed, and tissues to be examined microscopically were similarly fixed, processed, sectioned, and stained. Complete histopathologic examinations were performed on all rats and mice in the 0 and 2,100 ppm groups, and gross lesions were examined in rats and mice from all exposure groups. In addition, the kidneys of all male rats were also examined. Table 2 lists the tissues and organs examined.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP 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).

Supplemental Evaluations

Clinical Pathology Studies

During the 13-week studies, blood samples were collected from rats twice, on day 22 and at the end of the study, for hematology and clinical chemistry and from mice at the end of the study for hematology. Blood was taken from the retroorbital sinus of rats and mice anesthetized with a mixture of carbon dioxide and oxygen.

Samples for hematology determinations were collected in tubes containing sodium EDTA and analyzed with an Ortho ELT-8 Laser Hematology Counter (Ortho Instruments, Westwood, MA). Blood smears were stained with a Brecher's stain and counter stained with a modified Romanowsky stain. One hundred white cells were identified for differential leukocyte counts and the number of reticulocytes per 1,000 erythrocytes was counted on the same smear. Samples for clinical chemistry evaluations were collected in tubes without anticoagulant. They were allowed to clot, were centrifuged, and the serum was removed and analyzed with a Hitachi Automatic Chemistry Analyzer (Boehringer Mannheim, Indianapolis, IN).

Urine samples were collected from all rats for a 12-hour period twice during the 13-week study, on day 21 and during week 13. The rats were placed in individual metabolism cages for overnight urine collection. Food was withheld during the collection periods, although water was available *ad libitum*. Urine samples were collected in test tubes immersed in ice. The specific gravities of samples were determined using a refractometer (American Optical, Buffalo, NY). Urine samples were centrifuged for 5 minutes at 2000 rpm, the sediment was resuspended in 500 μL of the supernatant, and the suspension was spread on a slide and examined microscopically. Clinical pathology parameters evaluated in the 13-week studies are listed in Table 2.

Sperm Morphology and Vaginal Cytology Evaluations

At the end of the 13-week studies, sperm morphology and vaginal cytology evaluations were performed on 10 male and 10 female rats and mice exposed to 0, 540, 1,080, or 2,100 ppm according to methods outlined in the NTP's Sperm Motility Vaginal Cytology Evaluation protocol (NTP, 1984). For 7 consecutive days prior to sacrifice, the vaginal vaults of 10 female rats and mice per exposure group were lavaged, and the aspirated lavage fluid and cells were stained with Toluidine Blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, or metestrus).

Sperm motility was evaluated at necropsy in the following manner. The right testis and epididymis of 10 male rats and mice were weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five microscopic fields per slide by two independent observers.

Following completion of sperm motility estimates, each cauda epididymis was placed in phosphate-buffered saline solution. Caudae were finely minced and the tissue was incubated and then heat fixed at 65° C. Sperm density was determined microscopically with the aid of a hemocytometer and slides were prepared and stained with eosin for evaluation of sperm morphology.

TABLE 2
Experimental Design and Materials and Methods in the 18-Day and 13-Week Inhalation Studies of *t*-Butyl Alcohol

18-Day Studies	13-Week Studies
EXPERIMENTAL DESIGN	
Study Laboratory	
Battelle Columbus Laboratories, Columbus, OH	Battelle Columbus Laboratories, Columbus, OH
Size of Study Groups	
5 males and 5 females	10 males and 10 females
Exposure Concentrations/Duration	
Exposure concentrations: 0, 450, 900, 1,750, 3,500, and 7,000 ppm Duration: 6 hours plus T_{90} per day, 5 days per week (excluding one holiday) for 12 days	Exposure concentrations: 0, 136, 270, 540, 1,080, and 2,100 ppm Duration: 6 hours plus T_{90} per day, 5 days per week (excluding one holiday) for 13 weeks
Date of First Exposure	
Rats: 18 August 1986 Mice: 19 August 1986	Rats: 25-26 February 1987 Mice: 4-5 March 1987
Date of Last Exposure	
Rats: 3 September 1986 Mice: 4 September 1986	Rats: 27 or 28 May 1987 Mice: 3 or 4 June 1987
Necropsy Dates	
Rats: 4 September 1986 Mice: 5 September 1986	Rats: 28 or 29 May 1987 Mice: 4 or 5 June 1987
Type and Frequency of Observation	
Animals were observed twice daily, 7 days per week for mortality/morbidity. Body weights were recorded prior to the first exposure, on day 8, and at the end of the studies. Clinical observations were recorded twice daily.	Animals were observed twice daily, 7 days per week for mortality/morbidity. Body weights were recorded prior to the first exposure, weekly thereafter, and at the end of the studies. Clinical observations were recorded weekly.
Necropsy	
Complete necropsies were performed on all animals and the brain, heart, right kidney, liver, lung, right testis, and thymus were weighed.	Complete necropsies were performed on all animals and the brain, heart, right kidney, liver, lung, right testis, and thymus were weighed.

TABLE 2
Experimental Design and Materials and Methods in the 18-Day and 13-Week Inhalation Studies of *t*-Butyl Alcohol (continued)

18-Day Studies	13-Week Studies
EXPERIMENTAL DESIGN (continued)	
Histopathologic Examinations Complete histopathologic examinations were performed on all animals in the 0, 3,500, and 7,000 ppm groups. Tissues examined microscopically included: adrenal gland, bone and marrow, brain (three sections), clitoral gland (rats), esophagus, gallbladder (mice), heart, large intestine (cecum, colon, rectum), kidney, larynx, liver, lung, lymph nodes (bronchial, mandibular, mediastinal, and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland (rats), prostate gland, salivary gland, skin, small intestine (duodenum, jejunum, ileum), spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. Gross lesions were examined microscopically in all exposure groups.	Complete histopathologic examinations were performed on all animals in the 0 and 2,100 ppm groups. Tissues examined microscopically were the same as for the 18-day studies, with the addition of the clitoral and preputial glands of mice. Gross lesions were examined microscopically in all exposure groups. In addition, the kidneys of all groups of male rats were examined.
Supplemental Evaluations None	***Clinical pathology studies:*** Blood was collected from the retroorbital sinus of all rats on day 22 and at the end of the studies for hematology and clinical chemistry, and from all mice at the end of the studies for hematology. Urine was collected from rats on day 21 and at the end of the study. Hematology parameters evaluated included hematocrit, hemoglobin concentration, erythrocyte count, reticulocyte count, nucleated erythrocyte count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet count, and leukocyte count and differential. Clinical chemistry parameters evaluated in rats included alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, γ-glutamyltransferase, and bile salts. Urinalysis parameters evaluated in rats included urine volume, specific gravity, pH, and microscopic examination of sediment. ***Sperm morphology/vaginal cytology evaluations:*** Evaluations were performed on rats and mice in the 0, 540, 1,080, and 2,100 ppm groups. Male rats and mice were evaluated for necropsy body weights, reproductive tissue weights (right cauda, right epididymis, and right testis) and epididymal spermatozoal data (sperm density, morphology, and motility). Female rats and mice were evaluated for necropsy body weight, relative frequency of estrous stages, and estrous cycle length.

TABLE 2
Experimental Design and Materials and Methods in the 18-Day and 13-Week Inhalation Studies of *t*-Butyl Alcohol (continued)

18-Day Studies	13-Week Studies
ANIMALS AND ANIMAL MAINTENANCE	
Strain and Species	
F344/N rats $B6C3F_1$ mice	F344/N rats $B6C3F_1$ mice
Animal Source	
Taconic Laboratory and Animal Services, Germantown, NY	Taconic Laboratory and Animal Services, Germantown, NY
Time Held Before Study	
Rats: 10 days Mice: 11 days	13 days
Age When Study Began	
6 weeks	7 weeks
Age When Killed	
9 weeks	20 weeks
Method of Animal Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weight.	Same as 18-day studies
Diet	
NIH-07 open formula pellet diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, except during exposure periods; changed weekly.	NIH-07 Open Formula pellet diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, except during exposure and urine collection periods; changed weekly.
Chamber Environment	
Rats and mice were housed in individual cages in the exposure chambers for all studies. The temperature was maintained at 23.6° to 27.9° C and relative humidity at 47% to 76%, with 13-17 air changes per hour and 12 hours of fluorescent light per day.	Rats and mice were housed in individual cages. The temperature was maintained at 21° to 27.5° C and relative humidity at 28% to 76%, with 13-17 air changes per hour and 12 hours of fluorescent light per day.

STATISTICAL METHODS

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). Clinical chemistry, hematology, spermatid, and epididymal spermatozoal data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value. The extreme values chosen by the statistical test were subject to approval by NTP personnel. In addition, values indicated by the laboratory report as being inadequate due to technical problems were eliminated from the analysis.

Analysis of Vaginal Cytology Data

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across exposure levels.

GENETIC TOXICITY STUDIES

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1987). *t*-Butyl alcohol was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes

were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of *t*-butyl alcohol. In the absence of toxicity, 10,000 μg/plate was selected as the high dose. All assays were repeated.

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

Mouse Lymphoma Mutagenicity Test Protocol

The experimental protocol is presented in detail by McGregor *et al*. (1988). *t*-Butyl alcohol was supplied as a coded aliquot by Radian Corporation. The high dose of *t*-butyl alcohol was limited to 5,000 μg/mL. L5178Y mouse lymphoma cells were maintained at 37° C as suspension cultures in Fischer's medium supplemented with *l*-glutamine, sodium pyruvate, pluronic F68, antibiotics, and heat-inactivated horse serum; normal cycling time was approximately 10 hours. To reduce the number of spontaneously occurring trifluorothymidine-resistant cells, subcultures were exposed to medium containing THMG (thymidine, hypoxanthine, methotrexate, and glycine) for 1 day, to medium containing THG for 1 day, and to normal medium for 3 to 5 days. For cloning, the horse serum content was increased and Noble agar was added.

All treatment concentrations within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 mL medium. This volume included the S9 fraction in those experiments performed with metabolic activation. All cells were incubated with *t*-butyl alcohol for 4 hours, at which time the medium plus *t*-butyl alcohol was removed and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, cells were plated in medium and soft agar supplemented with trifluorothymidine (TFT) for selection of TFT-resistant ($TK^{-/-}$) cells; cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO_2 for 10 to 12 days. The test was initially performed

without S9. Because a clearly positive response was not obtained, the test was repeated using freshly prepared S9 from the livers of Aroclor 1254-induced or non-induced male F344/N rats.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Caspary *et al.* (1988). All data were evaluated statistically for trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for *t*-butyl alcohol to be considered positive, i.e., capable of inducing TFT resistance. A single significant response led to a "questionable" conclusion, and the absence of both a trend and peak response resulted in a "negative" call.

Chinese Hamster Ovary Cell Cytogenetics Protocols

Testing was performed as reported by Galloway *et al.* (1987). *t*-Butyl alcohol was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least four doses of *t*-butyl alcohol; 5,000 μg/mL was selected as the high dose. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

In the SCE test without S9, CHO cells were incubated for 26 hours with *t*-butyl alcohol in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing *t*-butyl alcohol was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with *t*-butyl alcohol, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no *t*-butyl alcohol, and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs per cell from each dose level.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with *t*-butyl alcohol for 9 to 9.5 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with *t*-butyl alcohol and S9 for 2 hours, after which the treatment medium was removed and the cells were

incubated for 9.5 to 10 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. One hundred first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

For the SCE data, statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P \leq 0.05$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.*, 1987). Ultimately, calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

Rat Bone Marrow Micronucleus Test Protocol

Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by *t*-butyl alcohol exposure. Male F344/N rats were injected intraperitoneally (three times at 24-hour intervals) with *t*-butyl alcohol dissolved in phosphate-buffered saline; the total dosing volume was 0.4 mL. Solvent control animals were injected with 0.4 mL of phosphate-buffered saline only. The positive control animals received cyclophosphamide. The rats were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells

obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes were scored for the frequency of micronucleated cells in each of five animals per dose group.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among polychromatic erythrocytes was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (Margolin *et al.*, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dose group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 13-week studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitude of those effects.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay can be found in MacGregor *et al.* (1990). Peripheral blood samples were obtained from male and female B6C3F_1 mice at the end of the 13-week drinking water study of *t*-butyl alcohol. Smears were immediately prepared and fixed in absolute methanol, stained with a chromatin-specific fluorescent dye mixture of Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983), and coded. Slides were scanned at 630× or 1,000× magnification using a semi-automated image analysis system to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes (NCEs) in up to 10 animals per dose group. Data were analyzed by the methods used for the bone marrow micronucleus test.

QUALITY ASSURANCE METHODS

The animal studies of *t*-butyl alcohol were performed in compliance with United States FDA Good Laboratory Practices regulations (21 CFR, Part 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.

RESULTS

18-DAY INHALATION STUDY IN F344/N RATS

All males and females exposed to 7,000 ppm died on day 2. All other rats survived to the end of the study (Table 3). Final mean body weights and mean body weight gains of 3,500 ppm males and females were significantly lower than those of the controls at the end of the 18-day study; males and females exposed to 3,500 ppm weighed 14% and 13% less than the controls, respectively.

TABLE 3
Survival and Body Weights of F344/N Rats in the 18-Day Inhalation Study of *t*-Butyl Alcohol

Exposure Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	90 ± 3	196 ± 5	105 ± 5	
450	5/5	91 ± 3	196 ± 4	105 ± 2	100
900	5/5	90 ± 3	204 ± 6	114 ± 4	104
1,750	5/5	91 ± 3	197 ± 5	105 ± 3	100
3,500	5/5	91 ± 3	168 ± 3**	77 ± 2**	86
7,000	0/5[c]	90 ± 2	—	—	—
Female					
0	5/5	87 ± 1	143 ± 2	56 ± 1	
450	5/5	88 ± 1	145 ± 2	58 ± 2	102
900	5/5	88 ± 2	146 ± 4	58 ± 3	102
1,750	5/5	88 ± 1	142 ± 2	54 ± 2	99
3,500	5/5	87 ± 2	125 ± 2**	37 ± 3**	87
7,000	0/5[c]	88 ± 2	—	—	—

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 18 days/number initially in group.
[b] Weights and weight changes are given as mean ± standard error. No data were calculated for groups with 100% mortality.
[c] Killed moribund on day 2.

All male and female rats in the 7,000 ppm group were moribund immediately following the first exposure to *t*-butyl alcohol and were therefore killed just before the second exposure was to begin. Clinical findings of toxicity in surviving males and females included ataxia, hyperactivity, and hypoactivity at exposure concentrations of 900 ppm and higher.

In 3,500 ppm males and females, thymus weights were decreased relative to those of the controls (Table A1). Other statistically significant organ weight differences were considered related to lower final mean body weights (in animals exposed to 3,500 ppm) or were considered random and unrelated to *t*-butyl alcohol exposure.

There were no treatment-related gross or microscopic findings in rats that died early or in those that survived to the end of the study.

13-WEEK INHALATION STUDY IN F344/N RATS

All rats survived to the end of the study, with the exception of one 135 ppm male accidentally killed during blood collection on day 22. Final mean body weights and body weight gains of all exposed groups of animals were similar to those of the controls (Table 4 and Figure 1).

TABLE 4
Survival and Body Weights of F344/N Rats in the 13-Week Inhalation Study of *t*-Butyl Alcohol

Exposure Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	131 ± 2	321 ± 7	190 ± 6	
135	9/10[c]	131 ± 3	319 ± 6	189 ± 4	100
270	10/10	127 ± 3	316 ± 7	189 ± 7	99
540	10/10	126 ± 2	327 ± 8	201 ± 6	102
1,080	10/10	129 ± 2	332 ± 5	202 ± 5	103
2,100	10/10	127 ± 2	328 ± 7	201 ± 6	102
Female					
0	10/10	109 ± 2	203 ± 3	93 ± 2	
135	10/10	109 ± 2	192 ± 4	84 ± 4	95
270	10/10	113 ± 5	201 ± 4	88 ± 4	99
540	10/10	113 ± 2	204 ± 2	92 ± 2	101
1,080	10/10	111 ± 2	203 ± 3	93 ± 2	100
2,100	10/10	108 ± 2	197 ± 3	89 ± 2	97

[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. Differences from the control group were not significant by Dunnett's test.
[c] Week of death: 4 (accidental death)

Exposure-related clinical signs in 2,100 ppm females were emaciation and hypoactivity, noted at one observation period only. All other clinical findings occurred sporadically and were not considered related to chemical administration.

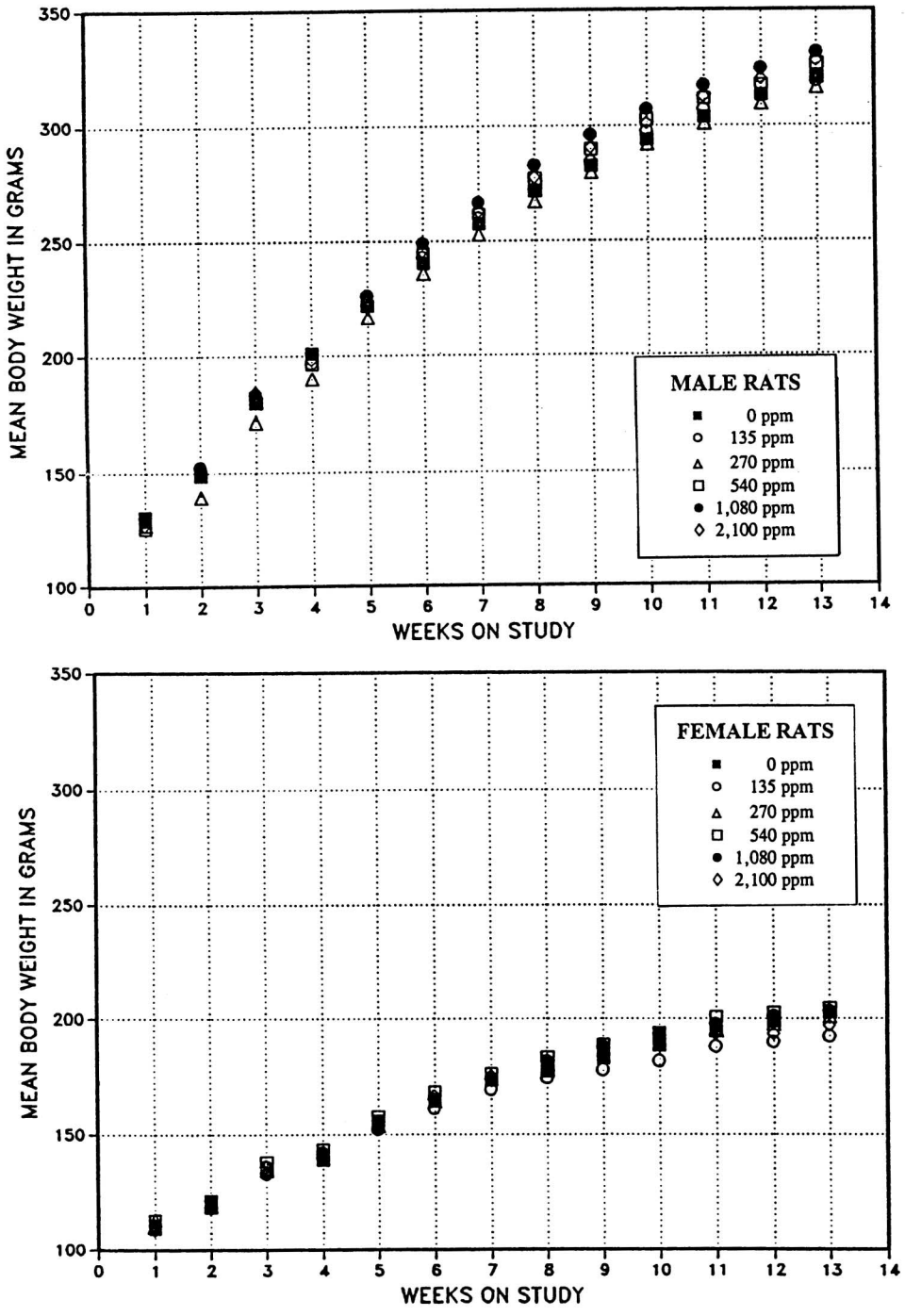


FIGURE 1
Body Weights of F344/N Rats Administered *t*-Butyl Alcohol by Inhalation for 13 Weeks

The hematology, clinical chemistry, and urinalysis data for rats in the 13-week inhalation study of *t*-butyl alcohol are listed in Tables 5, B1, B2, and B3. At week 13, a minimal anemia, evidenced by a minimal decrease in hematocrit values, hemoglobin concentrations, and erythrocyte counts, occurred in the 1,080 and 2,100 ppm male rats. Hemoglobin concentrations and/or hematocrit values also were minimally decreased in male rats in the 135, 270, and 540 ppm groups. Red blood cell variables were not decreased in the female rats. The mean cell volume, mean cell hemoglobin concentration, and reticulocyte and nucleated erythrocyte counts were not altered, indicating the anemia was normocytic, normochromic,

TABLE 5
Selected Clinical Pathology Data for F344/N Rats in the 13-Week Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Male						
n	10	9	10	10	10	10
Hematology						
Hematocrit (%)						
Day 22	50.2 ± 0.5	50.5 ± 0.5	50.1 ± 0.4	49.7 ± 0.4	49.4 ± 0.4	49.7 ± 0.5
Week 13	51.7 ± 0.5	50.3 ± 0.4	50.2 ± 0.4*	50.8 ± 0.5	49.2 ± 0.6**	49.3 ± 0.5**
Hemoglobin (g/dL)						
Day 22	15.9 ± 0.1	15.9 ± 0.1	15.8 ± 0.1	15.8 ± 0.1	15.6 ± 0.1	16.0 ± 0.2
Week 13	15.9 ± 0.2	15.4 ± 0.1*	15.4 ± 0.1*	15.6 ± 0.1*	15.0 ± 0.2**	15.1 ± 0.2**
Erythrocytes ($10^6/\mu$L)						
Day 22	8.86 ± 0.09	8.89 ± 0.09	8.81 ± 0.07	8.68 ± 0.08	8.72 ± 0.08	8.73 ± 0.10
Week 13	9.62 ± 0.10	9.41 ± 0.07	9.39 ± 0.07	9.47 ± 0.11	9.19 ± 0.12**	9.14 ± 0.09**
Clinical Chemistry						
Alkaline phosphatase (IU/L)						
Day 22	1,245 ± 22	1,311 ± 67	1,182 ± 21	1,156 ± 22*	1,086 ± 17**	1,074 ± 17**
Week 13	531 ± 15	504 ± 14	536 ± 17	490 ± 12	474 ± 12*	483 ± 10*
Urinalysis						
pH						
Day 21	6.77 ± 0.04	6.69 ± 0.06[b]	6.68 ± 0.03	6.55 ± 0.03**	6.50 ± 0.03**	6.42 ± 0.03**
Week 13	6.55 ± 0.03	6.51 ± 0.04	6.56 ± 0.04	6.56 ± 0.06	6.48 ± 0.03	6.34 ± 0.03**
Female						
n	10	10	10	10	10	10
Urinalysis						
pH						
Day 21	6.74 ± 0.05	6.73 ± 0.04	6.64 ± 0.05	6.60 ± 0.06[c]	6.52 ± 0.04**	6.46 ± 0.03**
Week 13	6.55 ± 0.04	6.60 ± 0.04	6.42 ± 0.05	6.40 ± 0.05	6.28 ± 0.05**	6.29 ± 0.10**

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=10
[c] n=9

and nonresponsive. A minimal to mild decrease in alkaline phosphatase activity occurred in the 540, 1,080, and 2,100 ppm male rats on day 22 and in the 1,080 and 2,100 ppm males at week 13. At week 13, a minimal decrease in urine pH occurred in the 2,100 ppm males and 1,080 and 2,100 ppm females. Other changes in clinical pathology variables were minimal and were not considered to be related to chemical exposure.

The absolute and relative right kidney weights of 1,080 and 2,100 ppm males were significantly greater than those of the controls (Tables 6 and A2). The relative right kidney weight of 2,100 ppm females and relative liver weights of 1,080 and 2,100 ppm females were significantly greater than those of the controls. Other statistically significant differences in organ weights of males were random and were not considered related to chemical administration.

TABLE 6
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Male						
n	10	9	10	10	10	10
Necropsy body wt	329 ± 8	327 ± 7	325 ± 8	332 ± 8	339 ± 5	331 ± 7
R. Kidney						
Absolute	1.206 ± 0.026	1.213 ± 0.032	1.180 ± 0.025	1.248 ± 0.035	1.341 ± 0.017**	1.324 ± 0.028**
Relative	3.68 ± 0.08	3.71 ± 0.04	3.64 ± 0.04	3.76 ± 0.06	3.96 ± 0.05**	4.00 ± 0.05**
Liver						
Absolute	13.273 ± 0.447	12.200 ± 0.324	13.005 ± 0.546	13.364 ± 0.615	14.571 ± 0.401	13.987 ± 0.329
Relative	40.37 ± 0.99	37.31 ± 0.45	39.93 ± 1.12	40.12 ± 1.05	43.11 ± 1.34	42.22 ± 0.66
Female						
n	10	10	10	10	10	10
Necropsy body wt	204 ± 3	196 ± 4	204 ± 4	206 ± 3	204 ± 4	195 ± 3
R. Kidney						
Absolute	0.817 ± 0.043	0.782 ± 0.020	0.821 ± 0.019	0.853 ± 0.014	0.831 ± 0.017	0.849 ± 0.012
Relative	4.00 ± 0.15	3.98 ± 0.06	4.03 ± 0.05	4.14 ± 0.04	4.09 ± 0.06	4.35 ± 0.03**
Liver						
Absolute	7.008 ± 0.139	6.977 ± 0.245	7.035 ± 0.158	7.210 ± 0.188	7.628 ± 0.253	7.295 ± 0.215
Relative	34.45 ± 0.49	35.49 ± 0.87	34.57 ± 0.65	35.03 ± 0.86	37.47 ± 1.04*	37.31 ± 0.73*

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

There were no treatment-related gross necropsy observations in exposed male or female rats and no microscopic findings in female rats. In male rats, an exposure-related increase in the severity of chronic nephropathy relative to the controls occurred in all exposed groups (Table 7). In control male rats, chronic nephropathy was a minimal lesion occurring in most animals and consisting of 1 to 3 scattered foci of regenerative tubules per kidney section. Regenerative foci were characterized by tubules with cytoplasmic basophilia, increased nuclear/cytoplasmic ratio, and occasionally thickened basement membranes and intraluminal protein casts. In exposed groups, the severity of nephropathy was increased as evidenced by an increased number of foci per section.

Sections of kidneys from male rats in the 0, 1,080, and 2,100 ppm groups (four per group) were stained by the Mallory-Heidenhain method for the presence of tubular hyaline droplet accumulation. There was no difference between control and exposed animals in the number, size, or shape of renal tubule hyaline droplets.

TABLE 7
Incidence and Severity of Chronic Nephropathy in Male F344/N Rats in the 13-Week Inhalation Study of *t*-Butyl Alcohol

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Kidney	10[a]	10	10	10	10	10
Nephropathy	9[b]	8	9	10	10	10
Severity	1.0[c]	1.4	1.4	1.6	1.9	2.0

[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected rats: 1 = minimal, 2 = mild

There were no significant differences in reproductive tissue parameters or estrous cycle characterization between exposed and control groups (Table C1).

18-DAY INHALATION STUDY IN B6C3F$_1$ MICE

All 7,000 ppm males and females died on day 2 and one male exposed to 3,500 ppm died on day 3 (Table 8). All other mice survived to the end of the study. Final mean body weights and body weight gains of exposed groups of mice were similar to those of the controls (Table 8).

TABLE 8
Survival and Body Weights of B6C3F$_1$ Mice in the 18-Day Inhalation Study of *t*-Butyl Alcohol

Exposure Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	20.8 ± 0.3	25.8 ± 0.5	5.0 ± 0.6	
450	5/5	21.0 ± 0.4	26.6 ± 0.7	5.6 ± 0.8	103
900	5/5	20.9 ± 0.3	25.7 ± 0.6	4.9 ± 0.8	100
1,750	5/5	21.0 ± 0.3	26.6 ± 1.1	5.6 ± 0.9	103
3,500	4/5[c]	20.9 ± 0.3	26.1 ± 0.6	5.3 ± 0.6	101
7,000	0/5[d]	20.8 ± 0.4	—	—	—
Female					
0	5/5	18.4 ± 0.4	22.8 ± 0.4	4.3 ± 0.4	
450	5/5	18.3 ± 0.4	21.9 ± 0.5	3.6 ± 0.5	96
900	5/5	18.2 ± 0.4	22.3 ± 0.3	4.1 ± 0.3	98
1,750	5/5	18.3 ± 0.4	22.6 ± 0.5	4.3 ± 0.7	99
3,500	5/5	18.2 ± 0.3	23.5 ± 0.5	5.3 ± 0.3	103
7,000	0/5[d]	18.5 ± 0.4	—	—	—

[a] Number of animals surviving at 18 days/number initially in group.
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. No data were calculated for groups with 100% mortality. Differences from the control group were not significant by Dunnett's test.
[c] Day of death: 3
[d] Killed moribund on day 2.

All male and female mice in the 7,000 ppm groups were moribund immediately following the first exposure to *t*-butyl alcohol and were therefore killed just before the second exposure was to begin. In 3,500 ppm males and females, animals were prostrate following the first exposure through day 3 of the study. Thereafter, clinical signs in these groups were observed predominantly post-exposure and included hypoactivity, ataxia, and rapid respiration. Hypoactivity, hyperactivity, ataxia, and urogenital wetness also occurred at lower incidences in mice exposed to 1,750 ppm.

The relative liver weight of 3,500 ppm males and absolute and relative liver weights of 3,500 ppm females were significantly greater than those of the controls, and the absolute and relative thymus weights of

3,500 ppm females were significantly lower than those of the controls (Table A3). Other statistically significant differences in organ weights of males were considered random and not related to chemical administration. There were no treatment-related gross findings or microscopic lesions present in mice that survived to the end of the study or in early deaths.

13-WEEK INHALATION STUDY IN B6C3F$_1$ MICE

Five males exposed to 1,080 ppm died during weeks 3 and 4 (Table 9). These deaths occurred in animals in adjacent cages and were thought to be due to a water or feed availability problem. Feed and water consumption data were not available to confirm this. The death of one 2,100 ppm male during week 7 was attributed to *t*-butyl alcohol exposure. The remaining mice survived to the end of the study. The initial mean body weight of 270 ppm males was significantly greater than that of the controls, but the mean body weight gain of this exposure group was significantly lower than that of the controls. The mean body weight gains of 1,080 and 2,100 ppm females and the final mean body weight of 2,100 ppm females were significantly lower than those of the controls. At the end of the 13-week study, females exposed to 2,100 ppm weighed 8% less than the controls (Table 9 and Figure 2).

TABLE 9
Survival and Body Weights of B6C3F$_1$ Mice in the 13-Week Inhalation Study of *t*-Butyl Alcohol

Exposure Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.2 ± 0.4	32.8 ± 0.8	9.6 ± 0.6	
135	10/10	22.1 ± 0.5	34.0 ± 1.0	12.0 ± 0.8*	104
270	10/10	25.2 ± 0.3**	32.2 ± 0.7	7.0 ± 0.4**	98
540	10/10	22.1 ± 0.6	32.9 ± 0.6	10.8 ± 0.4	100
1,080	5/10[c]	23.4 ± 0.4	31.6 ± 0.6	8.7 ± 0.8	96
2,100	9/10[d]	24.4 ± 0.3	32.7 ± 0.6	8.4 ± 0.4	100
Female					
0	10/10	18.5 ± 0.2	28.2 ± 0.6	9.7 ± 0.5	
135	10/10	18.8 ± 0.3	29.0 ± 0.6	10.2 ± 0.4	103
270	10/10	18.7 ± 0.3	27.2 ± 0.6	8.5 ± 0.5	97
540	10/10	19.0 ± 0.3	29.1 ± 0.9	10.2 ± 0.8	103
1,080	10/10	18.7 ± 0.3	26.6 ± 0.4	7.9 ± 0.2*	94
2,100	10/10	18.4 ± 0.2	25.8 ± 0.5**	7.4 ± 0.4**	92

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Week of death: 3, 3, 3, 4, 4
[d] Week of death: 7

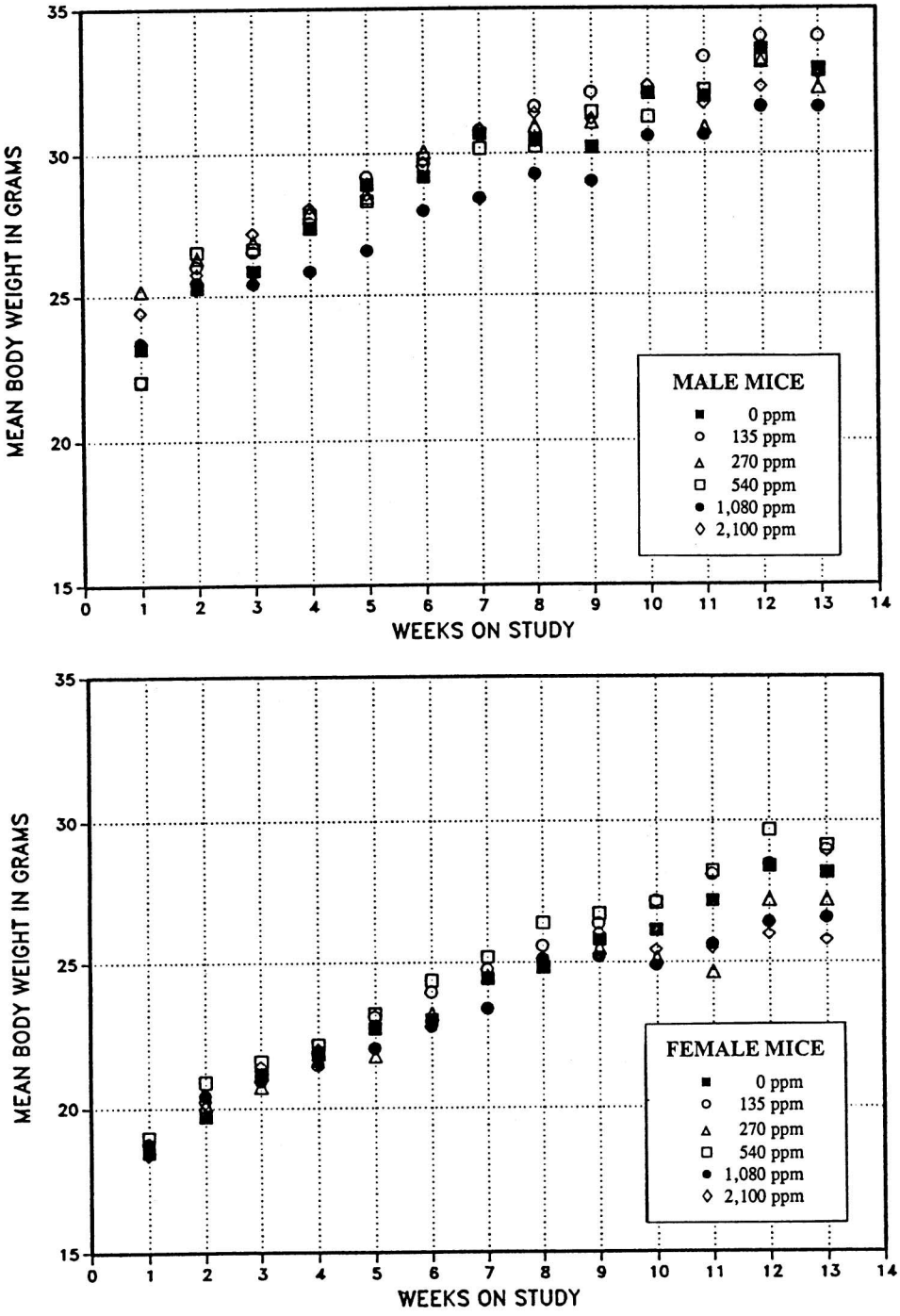


FIGURE 2
Body Weights of B6C3F$_1$ Mice Administered *t*-Butyl Alcohol by Inhalation for 13 Weeks

Clinical findings occurred sporadically and were not considered related to chemical administration.

The hematology data for mice in the 13-week inhalation study of *t*-butyl alcohol are listed in Table B4. At week 13, there was a marked increase in the segmented neutrophil count of 2,100 ppm male mice. Other changes in hematology variables were minimal, did not demonstrate a treatment relationship, and were not considered to be related to chemical exposure.

The relative liver weights of 1,080 and 2,100 ppm females were significantly greater than that of the controls. Other statistically significant organ weight differences in the 2,100 ppm females were considered to be related to the lower final mean body weights in this group (Table A4).

There were no treatment-related gross or microscopic observations in mice that survived to the end of the study. No lesions corresponding to the increased liver weights were found. No lesions to account for early deaths were present.

No significant differences occurred in the reproductive endpoints of exposed males (weight of testis, epididymis, and cauda; sperm motility, count, and morphology) or females (estrous cycle length or percentage of time spent in the various estrous stages) exposed to 540, 1,080, or 2,100 ppm *t*-butyl alcohol (Table C2).

GENETIC TOXICOLOGY

t-Butyl alcohol (100 to 10,000 μg/plate) did not induce mutations in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537 with or without induced rat or hamster liver S9 (Zeiger *et al.*, 1987; Table D1). Results of a mouse lymphoma cell mutation test were also considered to be negative, although a small increase in mutant colonies was observed in a single trial at the highest dose tested (5,000 μg/mL) in the absence of S9 (McGregor *et al.*, 1988; Table D2). McGregor *et al.* (1988) presented an additional trial conducted without S9 that showed no increase in mutant colonies at any of the doses tested; that trial is not included in Table D2 because it did not meet quality control standards for the assay. The two trials conducted with S9 are clearly negative. In cytogenetic tests with cultured Chinese hamster ovary cells, *t*-butyl alcohol at doses up to 5,000 μg/mL did not induce sister chromatid exchanges (Table D3) or chromosomal aberrations (Table D4), with or without S9. In the sister chromatid exchange test without S9, a weakly positive result was obtained in the first trial but it was not reproduced in the second trial. Neither trial conducted with S9 showed an increase in sister chromatid exchanges and the results of this test were considered negative. No cytotoxic effects were noted in the cultured Chinese hamster ovary cell experiments, with one exception. In the chromosomal aberrations test, the dose of 5,000 μg/mL in the second trial performed with S9 produced toxicity severe enough to allow only 13 cells to be analyzed for aberrations, rather than the usual 100 cells per dose point.

In vivo, no induction of micronuclei in polychromatic erythrocytes was observed in bone marrow cells of male rats receiving intraperitoneal injections of *t*-butyl alcohol (Table D5). No increase in the frequency of micronucleated normochromatic erythrocytes was observed in male or female mice administered *t*-butyl alcohol in drinking water for 13 weeks (Table D6). In addition, no effect on the percentage of polychromatic erythrocytes in the total erythrocyte population was noted, an indication that *t*-butyl alcohol was not toxic to bone marrow cells.

DISCUSSION

t-Butyl alcohol is widely used in cosmetics and in cleaning compounds and as a raw material in the production of the common gasoline additive methyl tertiary butyl ether. *t*-Butyl alcohol is also a major metabolite of methyl tertiary butyl ether. The National Cancer Institute nominated *t*-butyl alcohol for toxicity and carcinogenicity studies as a result of a review of chemicals found in drinking water. It was selected for study because of its large annual production and the potential for human exposure. Both inhalation and drinking water routes of administration were tested.

Similar to alcohols in general, the acute toxicity of *t*-butyl alcohol is related to alcohol intoxication and central nervous system depression. In the present 18-day studies, hypoactivity, ataxia, and prostration were observed in both rats and mice, and mortality occurred in both species at an exposure concentration of 7,000 ppm. The same clinical findings and mortality occurred at the highest exposure concentration (40 mg/mL) in the 13-week drinking water studies of *t*-butyl alcohol (NTP, 1995). Due to mortality at 7,000 ppm and decreased body weights or clinical signs of intoxication at 3,500 ppm, the highest exposure concentration selected for the 13-week studies was 1,700 ppm. During the course of the 13-week studies, a discrepancy between the target concentrations and actual concentrations generated was noted, such that the highest exposure concentration during these studies was 2,100 ppm.

Effects of *t*-butyl alcohol exposure on clinical pathology parameters in rats were of minimal severity and were limited to the 1,080 and 2,100 groups. A nonregenerative anemia and decreased serum alkaline phosphatase activity in exposed males could not be related to histopathologic or other toxicologic changes. In male mice, a marked increase in the number of segmented neutrophils was present in the 2,100 ppm group, consistent with an inflammatory process. There were, however, no gross or microscopic lesions to account for the neutrophilia.

Histopathologic treatment-related effects in the 13-week inhalation studies of *t*-butyl alcohol were limited to the kidney of male rats. Increases in kidney weight at the higher exposure concentrations corresponded microscopically to enhanced severity of chronic nephropathy. Chronic nephropathy is a common spontaneous lesion in male rats and frequently is exacerbated by chemical treatment. In the drinking water study of *t*-butyl alcohol, exacerbation of chronic nephropathy in male rats was also a treatment effect seen at

13 weeks (NTP, 1995). However, in that study the enhanced nephropathy was also associated with accumulation of renal tubule hyaline droplets (presumably α_{2u}-globulin), unlike the current study, in which special stains revealed no increase in protein droplets. The absence of protein accumulation in the present inhalation study, in spite of its presence in the previous drinking water study, is likely an exposure effect. For example, perchloroethylene administered at high doses by gavage has been shown to cause marked accumulation of hyaline droplets in rats. Inhalation exposure to this chemical at a concentration of 400 ppm does not induce this response, while protein accumulation occurs at a concentration of 1,000 ppm, suggesting that 400 ppm is below the threshold concentration required to induce this response (Green *et al.*, 1990). Increased severity of nephropathy in male rats can be a secondary effect of hyaline droplet accumulation and protein overload of tubule cells. The occurrence of exacerbated nephropathy in the present study, in which there was no confounding protein accumulation in the tubules, suggests that the mechanism of kidney cytotoxicity may be a direct effect of *t*-butyl alcohol and not limited to increased accumulation of protein. This would be further supported by the finding that slightly enhanced nephropathy also occurred in female rats of the drinking water study of *t*-butyl alcohol; this effect was likewise not complicated by protein overload of the tubule cells.

Chronic exposure to *t*-butyl alcohol in drinking water resulted in the occurrence of renal tubule neoplasms in male rats (NTP, 1995). Because the doses used in the 2-year study included those which caused hyaline droplet nephropathy in the 13-week drinking water study, the proposed mechanism of tumorigenesis under these conditions was cytotoxicity due to protein accumulation, leading to a sustained increase in renal cell proliferation and promotion of spontaneously initiated cells. However, a relationship between chemically enhanced chronic nephropathy, such as was seen in the current inhalation study, and the eventual development of renal tumors is less clear than that between hyaline droplet toxicity and renal carcinogenesis. Therefore, a prediction of the potential carcinogenic effect of *t*-butyl alcohol by the inhalation route based on comparison to the drinking water study is not warranted.

In the 13-week drinking water studies, the urinary bladder was also a target organ in rats and exhibited transitional cell hyperplasia and inflammation of the bladder mucosa (NTP, 1995). As discussed above for hyaline droplet nephropathy, it is likely that an exposure concentration sufficient to induce a similar bladder effect in the present inhalation study was precluded by other adverse effects. The urinary bladder and thyroid gland were found to be target organs in the 13-week and 2-year drinking water studies in mice. However, there was no pathologic or other evidence of toxicity in mice in the inhalation studies. No reproductive toxicity related to *t*-butyl alcohol inhalation exposure was evident in rats or mice.

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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 18-Day Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	450 ppm	900 ppm	1,750 ppm	3,500 ppm
Male					
n	5	5	5	5	5
Necropsy body wt	196 ± 5	196 ± 4	204 ± 6	197 ± 5	168 ± 3**
Brain					
Absolute	1.751 ± 0.012	1.724 ± 0.019	1.764 ± 0.023	1.699 ± 0.058	1.650 ± 0.025
Relative	8.97 ± 0.23	8.81 ± 0.21	8.68 ± 0.21	8.67 ± 0.35	9.84 ± 0.21
Heart					
Absolute	0.725 ± 0.020	0.777 ± 0.029	0.775 ± 0.024	0.768 ± 0.022	0.682 ± 0.041
Relative	3.72 ± 0.15	3.97 ± 0.13	3.82 ± 0.21	3.92 ± 0.12	4.06 ± 0.24
R. Kidney					
Absolute	0.819 ± 0.023	0.858 ± 0.023	0.911 ± 0.017*	0.884 ± 0.028	0.745 ± 0.026
Relative	4.19 ± 0.08	4.38 ± 0.12	4.48 ± 0.04	4.50 ± 0.06	4.43 ± 0.13
Liver					
Absolute	10.662 ± 0.262	11.240 ± 0.701	12.881 ± 0.539*	12.030 ± 0.383	9.619 ± 0.664
Relative	54.60 ± 1.37	57.29 ± 3.15	63.17 ± 1.27	61.23 ± 1.22	57.24 ± 3.69
Lung					
Absolute	1.144 ± 0.084	1.142 ± 0.054	1.329 ± 0.069	1.093 ± 0.056	1.095 ± 0.056
Relative	5.84 ± 0.34	5.83 ± 0.29	6.58 ± 0.52	5.60 ± 0.38	6.51 ± 0.29
R. Testis					
Absolute	1.144 ± 0.028	1.137 ± 0.013	1.163 ± 0.035	1.148 ± 0.034	1.115 ± 0.038
Relative	5.86 ± 0.21	5.80 ± 0.07	5.71 ± 0.11	5.84 ± 0.08	6.63 ± 0.11**
Thymus					
Absolute	0.482 ± 0.028	0.421 ± 0.031	0.466 ± 0.043	0.419 ± 0.026	0.312 ± 0.042**
Relative	2.47 ± 0.16	2.15 ± 0.17	2.28 ± 0.18	2.14 ± 0.13	1.87 ± 0.26

TABLE A1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 18-Day Inhalation Study of *t*-Butyl Alcohol (continued)

	0 ppm	450 ppm	900 ppm	1,750 ppm	3,500 ppm
Female					
n	5	5	5	5	5
Necropsy body wt	143 ± 2	145 ± 2	146 ± 4	142 ± 2	125 ± 2**
Brain					
Absolute	1.680 ± 0.029	1.675 ± 0.021	1.662 ± 0.021	1.625 ± 0.011	1.568 ± 0.027**
Relative	11.76 ± 0.19	11.53 ± 0.13	11.40 ± 0.29	11.50 ± 0.21	12.58 ± 0.10*
Heart					
Absolute	0.578 ± 0.028	0.587 ± 0.014	0.604 ± 0.021	0.539 ± 0.012	0.531 ± 0.043
Relative	4.04 ± 0.18	4.04 ± 0.13	4.14 ± 0.10	3.81 ± 0.12	4.24 ± 0.28
R. Kidney					
Absolute	0.592 ± 0.010	0.636 ± 0.012	0.632 ± 0.018	0.614 ± 0.017	0.573 ± 0.015
Relative	4.14 ± 0.05	4.38 ± 0.04	4.32 ± 0.07	4.35 ± 0.14	4.59 ± 0.05**
Liver					
Absolute	6.848 ± 0.342	6.752 ± 0.125	7.070 ± 0.302	6.472 ± 0.284	5.723 ± 0.329*
Relative	47.88 ± 2.14	46.49 ± 0.50	48.33 ± 1.22	45.82 ± 2.29	45.80 ± 1.93
Lung					
Absolute	0.935 ± 0.046	0.905 ± 0.063	1.013 ± 0.037	0.947 ± 0.029	0.858 ± 0.032
Relative	6.53 ± 0.24	6.22 ± 0.37	6.93 ± 0.20	6.70 ± 0.20	6.88 ± 0.22
Thymus					
Absolute	0.376 ± 0.026	0.392 ± 0.013	0.391 ± 0.015	0.327 ± 0.012	0.256 ± 0.020**
Relative	2.62 ± 0.17	2.70 ± 0.08	2.68 ± 0.07	2.31 ± 0.09	2.05 ± 0.16**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). No organ weights or organ-weight-to-body-weight ratios were calculated for animals receiving
8,400 ppm due to 100% mortality in this group.

TABLE A2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Male						
n	10	9	10	10	10	10
Necropsy body wt	329 ± 8	327 ± 7	325 ± 8	332 ± 8	339 ± 5	331 ± 7
Brain						
Absolute	1.932 ± 0.018	1.901 ± 0.017	1.896 ± 0.016	1.904 ± 0.011	1.871 ± 0.016*	1.892 ± 0.018
Relative	5.90 ± 0.12	5.83 ± 0.10	5.86 ± 0.11	5.76 ± 0.12	5.53 ± 0.08	5.72 ± 0.09
R. Epididymis						
Absolute	0.442 ± 0.004	0.487 ± 0.011*	0.475 ± 0.012	0.443 ± 0.008	0.445 ± 0.011	0.458 ± 0.009
Relative	1.35 ± 0.03	1.49 ± 0.05*	1.46 ± 0.03	1.34 ± 0.03	1.32 ± 0.03	1.39 ± 0.04
Heart						
Absolute	0.972 ± 0.019	0.941 ± 0.022	0.953 ± 0.028	0.955 ± 0.027	0.967 ± 0.011	1.012 ± 0.030
Relative	2.96 ± 0.04	2.88 ± 0.04	2.93 ± 0.05	2.88 ± 0.06	2.86 ± 0.04	3.05 ± 0.07
R. Kidney						
Absolute	1.206 ± 0.026	1.213 ± 0.032	1.180 ± 0.025	1.248 ± 0.035	1.341 ± 0.017**	1.324 ± 0.028**
Relative	3.68 ± 0.08	3.71 ± 0.04	3.64 ± 0.04	3.76 ± 0.06	3.96 ± 0.05**	4.00 ± 0.05**
Liver						
Absolute	13.273 ± 0.447	12.200 ± 0.324	13.005 ± 0.546	13.364 ± 0.615	14.571 ± 0.401	13.987 ± 0.329
Relative	40.37 ± 0.99	37.31 ± 0.45	39.93 ± 1.12	40.12 ± 1.05	43.11 ± 1.34	42.22 ± 0.66
Lung						
Absolute	1.743 ± 0.066	1.755 ± 0.029	1.743 ± 0.063	1.690 ± 0.057	1.851 ± 0.054	1.793 ± 0.064
Relative	5.31 ± 0.16	5.39 ± 0.12	5.37 ± 0.16	5.11 ± 0.19	5.47 ± 0.17	5.43 ± 0.21
R. Testis						
Absolute	1.465 ± 0.025	1.396 ± 0.017	1.363 ± 0.024*	1.446 ± 0.019	1.412 ± 0.030	1.464 ± 0.025
Relative	4.47 ± 0.09	4.28 ± 0.08	4.21 ± 0.07	4.37 ± 0.07	4.18 ± 0.11*	4.42 ± 0.04
Thymus						
Absolute	0.313 ± 0.024	0.317 ± 0.013	0.327 ± 0.011	0.335 ± 0.018[b]	0.320 ± 0.012	0.296 ± 0.012
Relative	0.95 ± 0.05	0.97 ± 0.05	1.01 ± 0.03	1.00 ± 0.04[b]	0.94 ± 0.02	0.89 ± 0.03

TABLE A2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of *t*-Butyl Alcohol (continued)

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Female						
n	10	10	10	10	10	10
Necropsy body wt	204 ± 3	196 ± 4	204 ± 4	206 ± 3	204 ± 4	195 ± 3
Brain						
Absolute	1.829 ± 0.018	1.786 ± 0.019	1.821 ± 0.016	1.831 ± 0.016	1.836 ± 0.021	1.768 ± 0.018
Relative	9.00 ± 0.10	9.11 ± 0.11	8.97 ± 0.18	8.90 ± 0.11	9.04 ± 0.19	9.06 ± 0.10
Heart						
Absolute	0.702 ± 0.017	0.678 ± 0.022	0.706 ± 0.011	0.734 ± 0.019	0.721 ± 0.011	0.698 ± 0.019
Relative	3.45 ± 0.06	3.45 ± 0.07	3.47 ± 0.05	3.57 ± 0.10	3.55 ± 0.06	3.57 ± 0.07
R. Kidney						
Absolute	0.817 ± 0.043	0.782 ± 0.020	0.821 ± 0.019	0.853 ± 0.014	0.831 ± 0.017	0.849 ± 0.012
Relative	4.00 ± 0.15	3.98 ± 0.06	4.03 ± 0.05	4.14 ± 0.04	4.09 ± 0.06	4.35 ± 0.03**
Liver						
Absolute	7.008 ± 0.139	6.977 ± 0.245	7.035 ± 0.158	7.210 ± 0.188	7.628 ± 0.253	7.295 ± 0.215
Relative	34.45 ± 0.49	35.49 ± 0.87	34.57 ± 0.65	35.03 ± 0.86	37.47 ± 1.04*	37.31 ± 0.73*
Lung						
Absolute	1.331 ± 0.045	1.315 ± 0.063	1.349 ± 0.051	1.397 ± 0.053	1.404 ± 0.023	1.358 ± 0.062
Relative	6.55 ± 0.22	6.69 ± 0.28	6.61 ± 0.18	6.79 ± 0.24	6.92 ± 0.16	6.95 ± 0.30
Thymus						
Absolute	0.272 ± 0.012	0.271 ± 0.010	0.269 ± 0.010	0.277 ± 0.012	0.253 ± 0.011	0.264 ± 0.007
Relative	1.34 ± 0.06	1.38 ± 0.04	1.32 ± 0.05	1.34 ± 0.05	1.24 ± 0.06	1.35 ± 0.03

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

TABLE A3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 18-Day Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	450 ppm	900 ppm	1,750 ppm	3,500 ppm
Male					
n	5	5	5	5	4
Necropsy body wt	25.8 ± 0.5	26.6 ± 0.7	25.7 ± 0.6	26.6 ± 1.1	26.1 ± 0.6
Brain					
Absolute	0.446 ± 0.004	0.450 ± 0.008	0.455 ± 0.007	0.449 ± 0.006	0.424 ± 0.004
Relative	17.27 ± 0.35	16.93 ± 0.32	17.70 ± 0.47	16.99 ± 0.72	16.27 ± 0.45
Heart					
Absolute	0.139 ± 0.005	0.140 ± 0.007	0.131 ± 0.004	0.134 ± 0.007	0.134 ± 0.005
Relative	5.38 ± 0.18	5.25 ± 0.14	5.07 ± 0.09	5.03 ± 0.19	5.14 ± 0.17
R. Kidney					
Absolute	0.256 ± 0.013	0.265 ± 0.011	0.244 ± 0.007	0.260 ± 0.007	0.233 ± 0.002
Relative	9.87 ± 0.33	9.98 ± 0.47	9.51 ± 0.33	9.82 ± 0.43	8.95 ± 0.19
Liver					
Absolute	1.600 ± 0.057	1.669 ± 0.074	1.568 ± 0.064	1.754 ± 0.105	1.852 ± 0.057
Relative	61.88 ± 1.59	62.62 ± 1.66	60.87 ± 1.48	65.69 ± 1.55	70.87 ± 1.17**
Lung					
Absolute	0.239 ± 0.017	0.202 ± 0.008	0.212 ± 0.008	0.203 ± 0.012	0.199 ± 0.011
Relative	9.25 ± 0.69	7.57 ± 0.24*	8.21 ± 0.20	7.65 ± 0.39*	7.63 ± 0.37
R. Testis					
Absolute	0.101 ± 0.003	0.101 ± 0.005	0.103 ± 0.003	0.098 ± 0.002	0.097 ± 0.002
Relative	3.91 ± 0.11	3.81 ± 0.25	3.99 ± 0.10	3.72 ± 0.18	3.73 ± 0.12
Thymus					
Absolute	0.057 ± 0.007	0.056 ± 0.002	0.058 ± 0.001	0.065 ± 0.007	0.041 ± 0.004
Relative	2.21 ± 0.29	2.09 ± 0.09	2.26 ± 0.03	2.42 ± 0.19	1.58 ± 0.19

TABLE A3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 18-Day Inhalation Study of *t*-Butyl Alcohol (continued)

	0 ppm	450 ppm	900 ppm	1,750 ppm	3,500 ppm
Female					
n	5	5	5	5	5
Necropsy body wt	22.8 ± 0.4	21.9 ± 0.5	22.3 ± 0.3	22.6 ± 0.5	23.5 ± 0.5
Brain					
Absolute	0.448 ± 0.015	0.454 ± 0.007	0.445 ± 0.011	0.458 ± 0.010	0.455 ± 0.014
Relative	19.68 ± 0.56	20.74 ± 0.42	19.95 ± 0.55	20.23 ± 0.25	19.45 ± 0.94
Heart					
Absolute	0.117 ± 0.002	0.117 ± 0.005	0.118 ± 0.003	0.123 ± 0.004	0.118 ± 0.002
Relative	5.16 ± 0.06	5.33 ± 0.10	5.29 ± 0.15	5.44 ± 0.13	5.03 ± 0.07
R. Kidney					
Absolute	0.177 ± 0.009	0.170 ± 0.008	0.169 ± 0.004	0.174 ± 0.004	0.178 ± 0.008
Relative	7.77 ± 0.35	7.76 ± 0.22	7.57 ± 0.16	7.67 ± 0.15	7.57 ± 0.21
Liver					
Absolute	1.368 ± 0.053	1.265 ± 0.069	1.393 ± 0.055	1.466 ± 0.055	1.754 ± 0.073**
Relative	60.06 ± 2.20	57.56 ± 1.87	62.33 ± 1.94	64.65 ± 1.26	74.56 ± 1.85**
Lung					
Absolute	0.180 ± 0.006	0.193 ± 0.011	0.191 ± 0.012	0.175 ± 0.010	0.182 ± 0.008
Relative	7.91 ± 0.38	8.78 ± 0.32	8.55 ± 0.46	7.71 ± 0.41	7.74 ± 0.32
Thymus					
Absolute	0.072 ± 0.004	0.069 ± 0.004	0.065 ± 0.001	0.075 ± 0.005	0.053 ± 0.004**
Relative	3.17 ± 0.20	3.17 ± 0.18	2.92 ± 0.06	3.30 ± 0.21	2.26 ± 0.19**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). No organ weights or organ-weight-to-body-weight ratios were calculated for animals receiving
8,400 ppm due to 100% mortality in this group.

TABLE A4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Male						
n	10	10	10	10	5	9
Necropsy body wt	34.9 ± 1.2	34.4 ± 1.1	33.3 ± 0.6	35.3 ± 0.7	32.6 ± 0.6	34.1 ± 0.8
Brain						
Absolute	0.444 ± 0.004	0.458 ± 0.007	0.460 ± 0.005	0.457 ± 0.006	0.442 ± 0.009	0.464 ± 0.006
Relative	12.86 ± 0.41	13.45 ± 0.44	13.84 ± 0.24	13.02 ± 0.36	13.59 ± 0.28	13.68 ± 0.42
R. Epididymis						
Absolute	0.043 ± 0.002	0.054 ± 0.005*	0.053 ± 0.003*	0.044 ± 0.001	0.044 ± 0.001	0.048 ± 0.002
Relative	1.25 ± 0.06	1.58 ± 0.13*	1.60 ± 0.09**	1.25 ± 0.03	1.35 ± 0.04	1.40 ± 0.05
Heart						
Absolute	0.169 ± 0.005	0.196 ± 0.013	0.197 ± 0.011	0.184 ± 0.005	0.165 ± 0.006	0.177 ± 0.008
Relative	4.87 ± 0.11	5.72 ± 0.33	5.96 ± 0.40*	5.22 ± 0.15	5.08 ± 0.21	5.18 ± 0.19
R. Kidney						
Absolute	0.315 ± 0.010	0.295 ± 0.009	0.311 ± 0.007	0.329 ± 0.007	0.285 ± 0.007	0.326 ± 0.011
Relative	9.06 ± 0.15	8.66 ± 0.32	9.34 ± 0.19	9.34 ± 0.25	8.78 ± 0.32	9.59 ± 0.29
Liver						
Absolute	1.673 ± 0.054	1.654 ± 0.071	1.739 ± 0.034	1.784 ± 0.046	1.543 ± 0.036	1.757 ± 0.047
Relative	48.24 ± 1.54	48.08 ± 1.13	52.40 ± 1.49	50.69 ± 1.36	47.47 ± 1.46	51.52 ± 0.71
Lung						
Absolute	0.259 ± 0.009	0.328 ± 0.019**	0.322 ± 0.008*	0.301 ± 0.017	0.305 ± 0.021	0.265 ± 0.012
Relative	7.51 ± 0.33	9.62 ± 0.60**	9.70 ± 0.34**	8.56 ± 0.51	9.37 ± 0.70	7.77 ± 0.33
R. Testis						
Absolute	0.117 ± 0.004	0.119 ± 0.002	0.114 ± 0.001	0.118 ± 0.003	0.114 ± 0.004	0.126 ± 0.004
Relative	3.39 ± 0.10	3.49 ± 0.09	3.43 ± 0.06	3.34 ± 0.06	3.52 ± 0.15	3.69 ± 0.10
Thymus						
Absolute	0.051 ± 0.003	0.052 ± 0.004	0.047 ± 0.004	0.052 ± 0.002	0.047 ± 0.006	0.044 ± 0.004
Relative	1.44 ± 0.07	1.50 ± 0.08	1.41 ± 0.11	1.46 ± 0.06	1.45 ± 0.16	1.31 ± 0.16

TABLE A4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of *t*-Butyl Alcohol (continued)

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Female						
n	10	10	10	10	10	10
Necropsy body wt	29.7 ± 0.6	31.1 ± 0.9	28.6 ± 0.8	30.8 ± 1.0	27.9 ± 0.5	26.8 ± 0.7*
Brain						
Absolute	0.469 ± 0.006	0.467 ± 0.005	0.465 ± 0.006	0.456 ± 0.004	0.458 ± 0.006	0.477 ± 0.005
Relative	15.83 ± 0.27	15.11 ± 0.48	16.37 ± 0.41	14.94 ± 0.46	16.53 ± 0.45	17.93 ± 0.61**
Heart						
Absolute	0.136 ± 0.005	0.140 ± 0.005	0.139 ± 0.003	0.139 ± 0.004	0.135 ± 0.003	0.136 ± 0.004
Relative	4.60 ± 0.15	4.51 ± 0.15	4.92 ± 0.20	4.53 ± 0.15	4.86 ± 0.14	5.09 ± 0.19
R. Kidney						
Absolute	0.202 ± 0.005	0.205 ± 0.005	0.213 ± 0.014	0.205 ± 0.004	0.203 ± 0.004	0.209 ± 0.002
Relative	6.82 ± 0.13	6.60 ± 0.19	7.46 ± 0.42	6.68 ± 0.15	7.32 ± 0.17	7.85 ± 0.22**
Liver						
Absolute	1.403 ± 0.039	1.421 ± 0.040	1.415 ± 0.036	1.469 ± 0.055	1.438 ± 0.028	1.522 ± 0.048
Relative	47.33 ± 1.31	45.67 ± 0.63	49.71 ± 1.14	47.75 ± 1.17	51.75 ± 1.35*	57.08 ± 2.15**
Lung						
Absolute	0.292 ± 0.013	0.272 ± 0.010	0.271 ± 0.014	0.281 ± 0.012	0.271 ± 0.008	0.279 ± 0.015
Relative	9.85 ± 0.41	8.77 ± 0.32	9.52 ± 0.42	9.16 ± 0.41	9.76 ± 0.30	10.54 ± 0.84
Thymus						
Absolute	0.063 ± 0.002	0.057 ± 0.003	0.058 ± 0.003	0.057 ± 0.003	0.055 ± 0.002	0.060 ± 0.004
Relative	2.13 ± 0.06	1.83 ± 0.08	2.04 ± 0.08	1.87 ± 0.10	1.97 ± 0.08	2.28 ± 0.19

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

APPENDIX B
HEMATOLOGY, CLINICAL CHEMISTRY, AND URINALYSIS RESULTS

TABLE B1
Hematology Data for F344/N Rats in the 13-Week Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Male						
n	10	9	10	10	10	10
Hematocrit (%)						
Day 22	50.2 ± 0.5	50.5 ± 0.5	50.1 ± 0.4	49.7 ± 0.4	49.4 ± 0.4	49.7 ± 0.5
Week 13	51.7 ± 0.5	50.3 ± 0.4	50.2 ± 0.4*	50.8 ± 0.5	49.2 ± 0.6**	49.3 ± 0.5**
Hemoglobin (g/dL)						
Day 22	15.9 ± 0.1	15.9 ± 0.1	15.8 ± 0.1	15.8 ± 0.1	15.6 ± 0.1	16.0 ± 0.2
Week 13	15.9 ± 0.2	15.4 ± 0.1*	15.4 ± 0.1*	15.6 ± 0.1*	15.0 ± 0.2**	15.1 ± 0.2**
Erythrocytes ($10^6/\mu L$)						
Day 22	8.86 ± 0.09	8.89 ± 0.09	8.81 ± 0.07	8.68 ± 0.08	8.72 ± 0.08	8.73 ± 0.10
Week 13	9.62 ± 0.10	9.41 ± 0.07	9.39 ± 0.07	9.47 ± 0.11	9.19 ± 0.12**	9.14 ± 0.09**
Reticulocytes ($10^6/\mu L$)						
Day 22	0.22 ± 0.02	0.20 ± 0.02	0.23 ± 0.01	0.20 ± 0.02	0.18 ± 0.02	0.18 ± 0.01
Week 13	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.02	0.12 ± 0.01	0.12 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)						
Day 22	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 13	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.05 ± 0.02
Mean cell volume (fL)						
Day 22	56.8 ± 0.1	57.0 ± 0.2	56.7 ± 0.3	57.3 ± 0.3	56.8 ± 0.3	56.9 ± 0.2
Week 13	53.8 ± 0.3	53.4 ± 0.2	53.5 ± 0.2	53.4 ± 0.2	53.4 ± 0.2	54.0 ± 0.2
Mean cell hemoglobin (pg)						
Day 22	18.0 ± 0.1	17.9 ± 0.1	17.9 ± 0.1	18.2 ± 0.1	17.9 ± 0.1	18.3 ± 0.1
Week 13	16.5 ± 0.1	16.3 ± 0.1	16.4 ± 0.1	16.4 ± 0.1	16.3 ± 0.1	16.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 22	31.7 ± 0.1	31.4 ± 0.2	31.5 ± 0.2	31.8 ± 0.1	31.6 ± 0.2	32.1 ± 0.1
Week 13	30.7 ± 0.1	30.6 ± 0.2	30.6 ± 0.1	30.7 ± 0.1	30.5 ± 0.2	30.6 ± 0.2
Platelets ($10^3/\mu L$)						
Day 22	594.0 ± 20.8	606.9 ± 9.7	638.7 ± 12.3	635.9 ± 10.7	657.4 ± 12.0*	613.7 ± 16.9
Week 13	570.0 ± 20.3	543.8 ± 15.2	532.4 ± 8.0	554.8 ± 22.2	580.3 ± 13.3	594.6 ± 33.0
Leukocytes ($10^3/\mu L$)						
Day 22	7.71 ± 0.36	7.48 ± 0.58	7.64 ± 0.53	6.90 ± 0.61	7.57 ± 0.26	6.71 ± 0.41
Week 13	8.40 ± 0.39	7.87 ± 0.68	7.22 ± 0.28	7.78 ± 0.45	8.75 ± 0.25	8.01 ± 0.58
Segmented neutrophils ($10^3/\mu L$)						
Day 22	0.52 ± 0.04	0.57 ± 0.08	0.45 ± 0.05	0.69 ± 0.08	0.63 ± 0.07	0.66 ± 0.07
Week 13	1.55 ± 0.15	1.20 ± 0.15	1.09 ± 0.08	1.30 ± 0.17	1.44 ± 0.12	1.83 ± 0.40
Lymphocytes ($10^3/\mu L$)						
Day 22	7.14 ± 0.35	6.87 ± 0.52	7.16 ± 0.52	6.20 ± 0.54	6.92 ± 0.23	6.00 ± 0.39
Week 13	6.78 ± 0.39	6.50 ± 0.56	6.05 ± 0.23	6.41 ± 0.40	7.19 ± 0.21	6.07 ± 0.41
Monocytes ($10^3/\mu L$)						
Day 22	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Week 13	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
Eosinophils ($10^3/\mu L$)						
Day 22	0.04 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.05 ± 0.02
Week 13	0.05 ± 0.03	0.13 ± 0.02*	0.04 ± 0.02	0.06 ± 0.02	0.10 ± 0.03	0.08 ± 0.03

TABLE B1
Hematology Data for F344/N Rats in the 13-Week Inhalation Study of *t*-Butyl Alcohol (continued)

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Female						
n						
Day 22	10	10	10	10	10	10
Week 13	10	10	10	10	9	10
Hematocrit (%)						
Day 22	51.0 ± 0.5	50.2 ± 0.4	50.8 ± 0.4	50.0 ± 0.6	50.3 ± 0.4	49.9 ± 0.4
Week 13	50.3 ± 0.5	50.9 ± 0.5	50.3 ± 0.4	50.3 ± 0.3	50.7 ± 0.6	50.2 ± 0.4
Hemoglobin (g/dL)						
Day 22	16.0 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	15.9 ± 0.2	16.0 ± 0.1	16.0 ± 0.2
Week 13	15.4 ± 0.2	15.5 ± 0.2	15.6 ± 0.2	15.5 ± 0.0	15.6 ± 0.2	15.4 ± 0.2
Erythrocytes ($10^6/\mu L$)						
Day 22	8.76 ± 0.09	8.74 ± 0.09	8.74 ± 0.09	8.65 ± 0.13	8.71 ± 0.09	8.70 ± 0.09
Week 13	8.71 ± 0.07	8.88 ± 0.10	8.79 ± 0.08	8.76 ± 0.05	8.85 ± 0.10	8.75 ± 0.07
Reticulocytes ($10^6/\mu L$)						
Day 22	0.16 ± 0.01	0.12 ± 0.01	0.10 ± 0.01*	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Week 13	0.13 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.02
Nucleated erythrocytes ($10^3/\mu L$)						
Day 22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 13	0.02 ± 0.01	0.06 ± 0.02	0.04 ± 0.02	0.07 ± 0.02	0.04 ± 0.01	0.05 ± 0.02
Mean cell volume (fL)						
Day 22	58.3 ± 0.3	57.5 ± 0.2	58.1 ± 0.3	57.7 ± 0.3	57.6 ± 0.3	57.2 ± 0.4
Week 13	57.7 ± 0.2	57.3 ± 0.3	57.3 ± 0.2	57.4 ± 0.2	57.4 ± 0.2	57.5 ± 0.3
Mean cell hemoglobin (pg)						
Day 22	18.2 ± 0.2	18.2 ± 0.1	18.4 ± 0.1	18.4 ± 0.1	18.3 ± 0.1	18.4 ± 0.1
Week 13	17.7 ± 0.1	17.5 ± 0.1	17.7 ± 0.1	17.6 ± 0.1	17.6 ± 0.1	17.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 22	31.3 ± 0.2	31.7 ± 0.2	31.5 ± 0.1	31.7 ± 0.1	31.7 ± 0.1*	32.1 ± 0.1**
Week 13	30.7 ± 0.2	30.5 ± 0.1	30.9 ± 0.1	30.7 ± 0.2	30.7 ± 0.1	30.6 ± 0.2
Platelets ($10^3/\mu L$)						
Day 22	605.4 ± 14.7	589.4 ± 16.2	597.3 ± 9.9	607.1 ± 14.3	584.9 ± 28.3	577.3 ± 27.1
Week 13	579.8 ± 17.0	532.7 ± 31.3	541.3 ± 11.8	535.5 ± 20.4	575.8 ± 19.1	583.3 ± 16.6
Leukocytes ($10^3/\mu L$)						
Day 22	6.75 ± 0.36	6.32 ± 0.34	6.61 ± 0.53	6.53 ± 0.64	5.89 ± 0.42	6.06 ± 0.38
Week 13	6.66 ± 0.39	6.35 ± 0.51	7.41 ± 0.50	6.99 ± 0.55	6.69 ± 0.28	5.37 ± 0.45
Segmented neutrophils ($10^3/\mu L$)						
Day 22	0.73 ± 0.11	0.72 ± 0.09	0.67 ± 0.07	0.55 ± 0.08	0.67 ± 0.11	0.73 ± 0.07
Week 13	0.95 ± 0.08	0.99 ± 0.11	1.31 ± 0.14	1.21 ± 0.10	1.39 ± 0.25	1.16 ± 0.15
Lymphocytes ($10^3/\mu L$)						
Day 22	5.96 ± 0.29	5.55 ± 0.32	5.87 ± 0.53	5.91 ± 0.62	5.17 ± 0.34	5.29 ± 0.35
Week 13	5.57 ± 0.32	5.31 ± 0.42	6.02 ± 0.42	5.69 ± 0.48	5.12 ± 0.18	4.15 ± 0.37
Monocytes ($10^3/\mu L$)						
Day 22	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
Week 13	0.04 ± 0.02	0.01 ± 0.01	0.00 ± 0.00*	0.01 ± 0.01	0.04 ± 0.02	0.00 ± 0.00
Eosinophils ($10^3/\mu L$)						
Day 22	0.05 ± 0.02	0.05 ± 0.02	0.08 ± 0.02	0.08 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
Week 13	0.09 ± 0.02	0.04 ± 0.01	0.08 ± 0.02	0.08 ± 0.02	0.14 ± 0.04	0.06 ± 0.02

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE B2
Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Male						
n	10	9	10	10	10	10
Alanine aminotransferase (IU/L)						
Day 22	41 ± 1	45 ± 2	39 ± 1	39 ± 1	38 ± 1	41 ± 2
Week 13	45 ± 1	50 ± 2	54 ± 5	47 ± 2	50 ± 2	52 ± 3
Alkaline phosphatase (IU/L)						
Day 22	1,245 ± 22	1,311 ± 67	1,182 ± 21	1,156 ± 22*	1,086 ± 17**	1,074 ± 17**
Week 13	531 ± 15	504 ± 14	536 ± 17	490 ± 12	474 ± 12*	483 ± 10*
Sorbitol dehydrogenase (IU/L)						
Day 22	38 ± 2	35 ± 2	31 ± 1*	32 ± 1	32 ± 1	30 ± 1*
Week 13	22 ± 1	25 ± 2	28 ± 3*	25 ± 1	27 ± 2*	26 ± 1
γ-Glutamyltransferase (IU/L)						
Day 22	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	1.0 ± 0.3
Week 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Bile salts (μmol/L)						
Day 22	20.2 ± 1.6	20.0 ± 1.5	19.3 ± 1.8	18.4 ± 2.7	20.2 ± 1.6	19.2 ± 1.4
Week 13	25.1 ± 3.9	28.0 ± 4.1	30.1 ± 4.3	26.9 ± 2.1	31.6 ± 2.0	33.4 ± 3.7
Female						
n	10	10	10	10	10	10
Alanine aminotransferase (IU/L)						
Day 22	40 ± 4	37 ± 1	35 ± 2	37 ± 1	37 ± 1	37 ± 1
Week 13	40 ± 1	42 ± 3	43 ± 2	43 ± 2	44 ± 4	44 ± 2
Alkaline phosphatase (IU/L)						
Day 22	971 ± 17	959 ± 29	900 ± 21	946 ± 25	903 ± 25	918 ± 21
Week 13	453 ± 21	500 ± 11	451 ± 13	429 ± 14	468 ± 19	455 ± 12
Sorbitol dehydrogenase (IU/L)						
Day 22	44 ± 3	40 ± 2	42 ± 2	39 ± 2	40 ± 2	38 ± 1
Week 13	23 ± 2	26 ± 1	26 ± 2	27 ± 1	26 ± 2	26 ± 1
γ-Glutamyltransferase (IU/L)						
Day 22	1.2 ± 0.4	1.3 ± 0.4	1.6 ± 0.5	1.2 ± 0.4	1.7 ± 0.5	1.6 ± 0.5
Week 13	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
Bile salts (μmol/L)						
Day 22	17.6 ± 2.2	15.5 ± 1.1	16.5 ± 0.9	16.0 ± 1.7	17.7 ± 1.4	15.7 ± 1.1
Week 13	18.5 ± 3.6	26.3 ± 5.0	32.2 ± 1.9*	26.3 ± 2.4	34.1 ± 2.7**	26.6 ± 2.0

* Significantly different ($P \le 0.05$) from the control group by Dunn's or Shirley's test
** $P \le 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE B3
Urinalysis Data for F344/N Rats in the 13-Week Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
n	10	10	10	10	10	10
Male						
Volume (mL/12 hours)						
Day 21	16.6 ± 1.5	17.9 ± 2.0	17.0 ± 2.3	16.2 ± 2.5	17.0 ± 2.1	22.0 ± 1.9
Week 13	7.2 ± 1.1	6.7 ± 0.9[b]	6.9 ± 1.3	6.8 ± 1.0	8.4 ± 1.6	8.1 ± 1.0
Specific gravity						
Day 21	1.009 ± 0.001	1.009 ± 0.001	1.011 ± 0.002	1.011 ± 0.002	1.013 ± 0.003	1.009 ± 0.001
Week 13	1.024 ± 0.003	1.021 ± 0.002[b]	1.023 ± 0.004	1.021 ± 0.002	1.022 ± 0.003	1.022 ± 0.001
pH						
Day 21	6.77 ± 0.04	6.69 ± 0.06	6.68 ± 0.03	6.55 ± 0.03**	6.50 ± 0.03**	6.42 ± 0.03**
Week 13	6.55 ± 0.03	6.51 ± 0.04[b]	6.56 ± 0.04	6.56 ± 0.06	6.48 ± 0.03	6.34 ± 0.03**
Female						
Volume (mL/12 hours)						
Day 21	13.5 ± 1.6	13.6 ± 1.7	9.5 ± 0.9	13.9 ± 2.9[b]	17.3 ± 1.5	16.7 ± 1.5
Week 13	5.2 ± 1.3	6.5 ± 1.8	4.9 ± 0.8	6.6 ± 2.5	5.5 ± 1.1	7.3 ± 2.3
Specific gravity						
Day 21	1.007 ± 0.001	1.009 ± 0.001	1.010 ± 0.001	1.009 ± 0.001[b]	1.007 ± 0.001	1.008 ± 0.001
Week 13	1.019 ± 0.003	1.018 ± 0.003	1.019 ± 0.002	1.022 ± 0.003	1.020 ± 0.003	1.019 ± 0.003
pH						
Day 21	6.74 ± 0.05	6.73 ± 0.04	6.64 ± 0.05	6.60 ± 0.06[b]	6.52 ± 0.04**	6.46 ± 0.03**
Week 13	6.55 ± 0.04	6.60 ± 0.04	6.42 ± 0.05	6.40 ± 0.05	6.28 ± 0.05**	6.29 ± 0.10**

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

TABLE B4
Hematology Data for B6C3F$_1$ Mice in the 13-Week Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Male						
n	10	10	10	10	5	9
Hematocrit (%)	56.7 ± 0.8[b]	59.5 ± 0.5	57.3 ± 1.1	56.2 ± 1.2	61.4 ± 1.1*	58.7 ± 1.0
Hemoglobin (g/dL)	16.6 ± 0.4	17.4 ± 0.2	16.8 ± 0.4	16.6 ± 0.4	18.0 ± 0.3	17.3 ± 0.2
Erythrocytes (10^6/μL)	10.79 ± 0.18	11.17 ± 0.14	10.70 ± 0.31	10.51 ± 0.26	11.62 ± 0.24	11.01 ± 0.12
Reticulocytes (10^6/μL)	0.15 ± 0.01	0.13 ± 0.01	0.21 ± 0.03	0.16 ± 0.02	0.16 ± 0.02	0.13 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.01 ± 0.01[b]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Mean cell volume (fL)	52.5 ± 0.2	53.3 ± 0.3	53.6 ± 0.7	53.6 ± 0.3	52.8 ± 0.2	53.3 ± 0.4
Mean cell hemoglobin (pg)	15.4 ± 0.2	15.6 ± 0.1	15.7 ± 0.2	15.8 ± 0.2	15.5 ± 0.1	15.7 ± 0.1
Mean cell hemoglobin concentration (g/dL)	29.3 ± 0.4	29.2 ± 0.2	29.2 ± 0.2	29.5 ± 0.3	29.3 ± 0.1	29.5 ± 0.3
Platelets (10^3/μL)	779.3 ± 34.8	719.9 ± 40.6	724.5 ± 37.8	695.1 ± 29.0	637.8 ± 76.1	743.2 ± 31.8
Leukocytes (10^3/μL)	4.98 ± 0.60[b]	5.14 ± 0.36	6.48 ± 0.91	5.66 ±0.47	4.88 ± 0.75	5.66 ± 0.47
Segmented neutrophils (10^3/μL)	0.65 ± 0.12[b]	0.72 ± 0.11	0.98 ± 0.20	0.73 ± 0.10	0.66 ± 0.10	2.07 ± 0.32**
Lymphocytes (10^3/μL)	4.22 ± 0.53[b]	4.34 ± 0.29	5.36 ± 0.79	4.84 ± 0.41	4.06 ± 0.65	3.38 ± 0.55
Monocytes (10^3/μL)	0.00 ± 0.00[b]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01*	0.00 ± 0.00
Eosinophils (10^3/μL)	0.11 ± 0.03[b]	0.08 ± 0.02	0.14 ± 0.03	0.09 ± 0.03	0.16 ± 0.04	0.10 ± 0.03

TABLE B4
Hematology Data for B6C3F$_1$ Mice in the 13-Week Inhalation Study of *t*-Butyl Alcohol (continued)

	0 ppm	135 ppm	270 ppm	540 ppm	1,080 ppm	2,100 ppm
Female						
n	10	10	10	9	10	10
Hematocrit (%)	57.6 ± 0.8	59.2 ± 0.4	58.1 ± 0.7	58.4 ± 0.6	59.4 ± 0.3	59.9 ± 0.8
Hemoglobin (g/dL)	16.7 ± 0.3	17.2 ± 0.2	16.8 ± 0.2	17.1 ± 0.2	17.3 ± 0.2	17.5 ± 0.2
Erythrocytes ($10^6/\mu$L)	10.79 ± 0.17	11.04 ± 0.10	10.91 ± 0.14	10.98 ± 0.10	11.11 ± 0.08	11.12 ± 0.10
Reticulocytes ($10^6/\mu$L)	0.16 ± 0.02	0.13 ± 0.02	0.16 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.14 ± 0.01
Nucleated erythrocytes ($10^3/\mu$L)	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	53.5 ± 0.2	53.7 ± 0.4	53.2 ± 0.4	53.1 ± 0.3	53.7 ± 0.3	53.9 ± 0.4
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.6 ± 0.1	15.4 ± 0.1	15.5 ± 0.1	15.5 ± 0.1	15.7 ± 0.1
Mean cell hemoglobin concentration (g/dL)	29.1 ± 0.2	29.0 ± 0.3	28.9 ± 0.2	29.2 ± 0.2	29.1 ± 0.3	29.2 ± 0.2
Platelets ($10^3/\mu$L)	640.5 ± 26.5	606.9 ± 30.6	634.4 ± 28.6	615.7 ± 47.1	596.4 ± 19.3	586.0 ± 33.4
Leukocytes ($10^3/\mu$L)	3.55 ± 0.49	4.52 ± 0.32	4.10 ± 0.26	4.00 ± 0.32	4.01 ± 0.21	4.68 ± 0.28
Segmented neutrophils ($10^3/\mu$L)	0.47 ± 0.10	0.54 ± 0.08	0.43 ± 0.07	0.47 ± 0.05	0.46 ± 0.05	0.66 ± 0.05
Lymphocytes ($10^3/\mu$L)	3.06 ± 0.40	3.91 ± 0.27	3.62 ± 0.27	3.43 ± 0.29	3.46 ± 0.18	3.94 ± 0.26
Monocytes ($10^3/\mu$L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[c]	0.00 ± 0.00	0.00 ± 0.00
Eosinophils ($10^3/\mu$L)	0.03 ± 0.01	0.08 ± 0.03	0.05 ± 0.02	0.10 ± 0.02	0.09 ± 0.03	0.08 ± 0.03

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=10

APPENDIX C
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE C1
Summary of Reproductive Tissue Evaluations and Estrous Cycle Characterization for F344/N Rats in the 13-Week Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	540 ppm	1,080 ppm	2,100 ppm
Male				
n	10	10	10	10
Weights (g)				
Necropsy body weight	329 ± 8	332 ± 8	339 ± 5	331 ± 7
Right cauda epididymis	0.163 ± 0.004	0.167 ± 0.004	0.171 ± 0.006	0.171 ± 0.004
Right epididymis	0.442 ± 0.004	0.443 ± 0.008	0.445 ± 0.011	0.458 ± 0.009
Right testis	1.47 ± 0.03	1.45 ± 0.02	1.41 ± 0.03	1.46 ± 0.03
Epididymal spermatozoal measurements				
Motility (%)	66.38 ± 1.53	65.70 ± 1.69	66.01 ± 2.16	67.76 ± 2.80
Abnormality (%)	1.020 ± 0.128	1.140 ± 0.280	0.940 ± 0.146	1.160 ± 0.129
Concentration (10^6/g cauda epididymal tissue)	534 ± 29	479 ± 34	530 ± 22	540 ± 34
Female				
n	10	10	10	10
Necropsy body weight (g)	204 ± 3	206 ± 3	204 ± 4	195 ± 3
Estrous cycle length (days)	4.80 ± 0.13	4.60 ± 0.16	4.90 ± 0.18	5.00 ± 0.15
Estrous stages (% of cycle)				
Diestrus	40.0	38.6	42.9	38.6
Proestrus	20.0	14.3	14.3	20.0
Estrus	22.9	24.3	22.9	21.4
Metestrus	15.7	20.0	17.1	20.0
Uncertain diagnoses	1.4	2.9	2.9	0.0

[a] All data except the estrous stages are presented as mean ± standard error; differences from the control group are not significant by Dunnett's test (body weights only) or Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the controls in the relative length of time spent in the estrous stages.

TABLE C2
Summary of the Reproductive Tissue Evaluations and Estrous Cycle Characterization for B6C3F$_1$ Mice in the 13-Week Inhalation Study of *t*-Butyl Alcohol[a]

	0 ppm	540 ppm	1,080 ppm	2,100 ppm
Male				
n	10	10	5	9
Weights (g)				
Necropsy body weight	34.9 ± 1.2	35.3 ± 0.7	32.6 ± 0.6	34.1 ± 0.8
Right cauda epididymis	0.015 ± 0.001	0.016 ± 0.001	0.017 ± 0.001	0.017 ± 0.001
Right epididymis	0.043 ± 0.002	0.044 ± 0.001	0.044 ± 0.001	0.048 ± 0.002
Right testis	0.117 ± 0.004	0.118 ± 0.003	0.114 ± 0.004	0.126 ± 0.004
Epididymal spermatozoal measurements				
Motility (%)	69.93 ± 1.80	65.90 ± 1.56	65.52 ± 2.13	66.04 ± 3.04
Abnormality (%)	1.90 ± 0.13	1.86 ± 0.25	1.92 ± 0.19	2.24 ± 0.21
Concentration (10^6/g cauda epididymal tissue)	571 ± 71	807 ± 53	657 ± 56	700 ± 84
Female				
n	10	10	10	10
Necropsy body weight (g)	29.7 ± 0.6	30.8 ± 1.0	27.9 ± 0.5	26.8 ± 0.7*
Estrous cycle length (days)	4.50 ± 0.17	4.38 ± 0.18[b]	4.11 ± 0.20[c]	4.29 ± 0.18[d]
Estrous stages (% of cycle)				
Diestrus	24.3	27.1	31.4	35.7
Proestrus	22.9	20.0	17.1	22.9
Estrus	31.4	27.1	25.7	22.9
Metestrus	21.4	18.6	22.9	12.9
Uncertain diagnoses	0.0	7.1	2.9	5.7

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

[a] All data except the estrous stages are presented as mean ± standard error; differences from the control group are not significant by Dunnett's test (male body weights only) or Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from controls in the relative length of time spent in the estrous stages.

[b] Estrous cycle was longer than 7 days or was unclear in 2 of 10 animals.

[c] Estrous cycle was longer than 7 days or was unclear in 1 of 10 animals.

[d] Estrous cycle was longer than 7 days or was unclear in 3 of 10 animals.

APPENDIX D
GENETIC TOXICOLOGY

TABLE D1
Mutagenicity of *t*-Butyl Alcohol in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	121 ± 2.2	70 ± 6.2	136 ± 5.2	98 ± 3.4	133 ± 5.9	83 ± 2.2
	100	97 ± 13.9	69 ± 7.5	125 ± 15.0	83 ± 1.7	109 ± 8.1	85 ± 8.0
	333	98 ± 9.1	75 ± 2.9	113 ± 1.2	85 ± 4.5	134 ± 8.5	91 ± 3.5
	1,000	92 ± 9.0	92 ± 13.0	122 ± 8.7	85 ± 3.8	117 ± 4.9	84 ± 5.2
	3,333	110 ± 4.7	90 ± 6.9	116 ± 12.3	86 ± 4.9	114 ± 11.8	78 ± 4.3
	10,000	99 ± 9.0	86 ± 4.1	114 ± 3.8	75 ± 1.7	118 ± 8.1	82 ± 4.8
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		585 ± 40.5	495 ± 18.9	1,915 ± 139.3	2,124 ± 40.9	1,001 ± 112.9	1,372 ± 67.7
TA1535	0	5 ± 0.3	5 ± 0.9	7 ± 1.5	8 ± 2.1	7 ± 1.3	7 ± 3.5
	100	6 ± 0.3	8 ± 0.9	8 ± 0.9	9 ± 1.5	9 ± 0.3	11 ± 1.2
	333	4 ± 0.9	8 ± 0.9	6 ± 0.7	10 ± 1.7	6 ± 1.8	11 ± 2.6
	1,000	4 ± 0.7	6 ± 1.2	4 ± 0.9	11 ± 2.9	8 ± 0.9	7 ± 0.6
	3,333	4 ± 0.9	8 ± 1.0	7 ± 2.3	13 ± 3.2	6 ± 1.3	10 ± 0.9
	10,000	6 ± 1.0	9 ± 1.5	9 ± 1.5	13 ± 1.7	8 ± 0.3	11 ± 2.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		623 ± 19.8	526 ± 84.8	160 ± 11.1	86 ± 6.2	51 ± 0.7	90 ± 10.7
TA1537	0	3 ± 0.7	4 ± 1.2	8 ± 0.9	7 ± 0.6	11 ± 1.5	6 ± 2.5
	100	2 ± 1.9	7 ± 1.3	6 ± 2.0	7 ± 0.6	5 ± 1.3	6 ± 2.6
	333	3 ± 0.7	6 ± 1.8	5 ± 1.3	8 ± 1.2	4 ± 0.6	9 ± 3.2
	1,000	3 ± 1.2	6 ± 2.0	5 ± 0.3	10 ± 0.0	4 ± 1.3	7 ± 0.6
	3,333	2 ± 2.3	8 ± 0.6	5 ± 1.8	9 ± 4.4	4 ± 0.7	10 ± 1.5
	10,000	1 ± 0.9	6 ± 2.0	5 ± 2.0	6 ± 2.0	7 ± 0.6	10 ± 0.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		247 ± 99.6	101 ± 40.4	38 ± 3.0	39 ± 3.5	73 ± 11.8	108 ± 11.9
TA98	0	13 ± 2.5	13 ± 3.2	18 ± 3.8	18 ± 3.0	20 ± 0.3	20 ± 1.2
	100	12 ± 1.5	9 ± 2.3	19 ± 2.9	17 ± 4.4	16 ± 2.4	14 ± 0.3
	333	17 ± 1.9	8 ± 2.3	17 ± 3.5	12 ± 1.9	15 ± 1.5	15 ± 0.6
	1,000	9 ± 2.6	18 ± 3.7	14 ± 0.9	12 ± 1.7	21 ± 2.6	14 ± 2.9
	3,333	15 ± 5.8	10 ± 0.6	18 ± 0.7	15 ± 1.9	17 ± 1.5	13 ± 2.0
	10,000	10 ± 1.3	8 ± 0.6	17 ± 1.0	15 ± 2.3	18 ± 3.0	9 ± 1.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		234 ± 36.7	102 ± 7.9	1,442 ± 86.7	1,528 ± 84.1	836 ± 161.8	590 ± 70.0

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

TABLE D2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by *t*-Butyl Alcohol[a]

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction[c]
-S9						
Medium		62	100	144	78	
		60	96	160	90	
		67	111	207	103	
		54	93	157	98	92
Methyl methanesulfonate	15	20	22	369	605	
		23	25	337	499	552*
t-Butyl alcohol	1,000	55	112	153	93	
		62	91	185	100	96
	2,000	41	87	160	130	
		53	98	198	124	127
	3,000	62	81	257	139	
		55	90	191	115	127
	4,000	50	78	191	126	
		51	78	243	158	142
	5,000	58	75	275	157	
		52	71	227	146	152*
+S9						
Trial 1						
Medium		71	101	110	52	
		80	108	135	56	
		73	91	87	40	49
Methylcholanthrene	2.5	50	41	506	335	
		51	38	480	317	326*
t-Butyl alcohol	1,000	95	106	122	43	
		66	92	95	48	45
	2,000	77	101	96	42	
		92	108	124	45	43
	3,000	74	92	83	37	
		82	99	110	45	41
	4,000	71	115	110	52	
	5,000	81	102	100	41	
		84	118	97	39	40

TABLE D2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by *t*-Butyl Alcohol
(continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9 (continued)						
Trial 2						
Medium		103	105	67	22	
		93	104	34	12	
		70	95	37	18	
		92	96	52	19	18
Methylcholanthrene	2.5	77	33	531	231	
		67	38	461	231	231*
t-Butyl alcohol	2,000	62	105	61	33	
		80	94	44	18	26
	3,000	78	90	45	19	
		71	90	69	33	26
	4,000	79	95	33	14	
		80	90	47	20	17
	5,000	97	101	69	24	
		70	88	58	28	26

* Significant positive response ($P \leq 0.05$)
[a] Study performed at Inveresk Research International. The experimental protocol and these data are presented in McGregor *et al.* (1988). All doses were tested in triplicate; the average of the three tests is presented in the table.
[b] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/10^6 cells treated); MF = mutant fraction.
[c] Mean from three replicate plates of approximately 10^6 cells each

TABLE D3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *t*-Butyl Alcohol[a]

Compound	Dose μg/mL	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Trial 1								
Summary: Weakly positive								
Medium		50	1,039	419	0.40	8.4	26.0	
Mitomycin-C	0.001	50	1,039	661	0.63	13.2	26.0	57.76
	0.010	10	209	556	2.66	55.6	26.0	559.68
t-Butyl alcohol	160	50	1,045	444	0.42	8.9	26.0	5.36
	500	50	1,047	457	0.43	9.1	26.0	8.24
	1,600	50	1,046	486	0.46	9.7	26.0	15.21
	5,000	50	1,049	509	0.48	10.2	26.0	20.32*
					P < 0.001[c]			
Trial 2								
Summary: Negative								
Medium		50	1,040	430	0.41	8.6	26.0	
Mitomycin-C	0.001	50	1,048	1,287	1.22	25.7	26.0	197.02
	0.010	10	210	674	3.20	67.4	26.0	676.26
t-Butyl alcohol	2,000	50	1,037	437	0.42	8.7	26.0	1.92
	3,000	50	1,039	433	0.41	8.7	26.0	0.79
	4,000	50	1,046	478	0.45	9.6	26.0	10.52
	5,000	50	1,040	453	0.43	9.1	26.0	5.35
					P = 0.104			
+S9								
Trial 1								
Summary: Negative								
Medium		50	1,035	474	0.45	9.5	26.0	
Cyclophosphamide	0.3	50	1,041	606	0.58	12.1	26.0	27.11
	2.0	10	210	322	1.53	32.2	26.0	234.81
t-Butyl alcohol	160	50	1,037	459	0.44	9.2	26.0	-3.35
	500	50	1,048	438	0.41	8.8	26.0	-8.74
	1,600	50	1,040	452	0.43	9.0	26.0	-5.10
	5,000	50	1,047	400	0.38	8.0	26.0	-16.58
					P = 0.994			

TABLE D3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *t*-Butyl Alcohol (continued)

Compound	Dose μg/mL	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome (%)
+S9 (continued)								
Trial 2								
Summary: Negative								
Medium		50	1,038	469	0.45	9.4	26.0	
Cyclophosphamide	0.3	50	1,044	622	0.59	12.4	26.0	31.86
	2.0	10	210	319	1.51	31.9	26.0	236.20
t-Butyl alcohol	2,000	50	1,047	505	0.48	10.1	26.0	6.75
	3,000	50	1,043	454	0.43	9.1	26.0	-3.66
	4,000	50	1,042	448	0.42	9.0	26.0	-4.85
	5,000	50	1,037	482	0.46	9.6	26.0	2.87
					P=0.715			

* Positive (≥20% increase over solvent control)
[a] Study performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Galloway *et al.* (1987). SCE = sister chromatid exchange; BrdU = bromodeoxyuridine.
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c] Significance of SCEs/chromosome tested by linear regression trend test vs. log of the dose

TABLE D4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *t*-Butyl Alcohol[a]

-S9					+S9				
Dose (μg/mL)	**Total Cells**	**No. of Abs**	**Abs/ Cell**	**Cells with Abs (%)**	**Dose (μg/mL)**	**Total Cells**	**No. of Abs**	**Abs/ Cell**	**Cells with Abs (%)**
Trial 1 - Harvest time: 11.0 hours Summary: Negative					**Trial 1** - Harvest time: 11.5 hours Summary: Equivocal				
Medium					Medium				
	100	3	0.03	3.0		100	0	0.00	0.0
Mitomycin-C					Cyclophosphamide				
0.25	100	42	0.42	32.0	15	100	20	0.20	19.0
1.00	50	27	0.54	28.0	50	100	61	0.61	42.0
t-Butyl alcohol					*t*-Butyl Alcohol				
160	100	3	0.03	3.0	160	100	4	0.04	4.0
500	100	4	0.04	4.0	500	100	1	0.01	1.0
1,600	100	1	0.01	1.0	1,600	100	3	0.03	3.0
5,000	100	3	0.03	3.0	5,000	100	6	0.06	6.0*
				P=0.651[b]					P=0.017
Trial 2 - Harvest time: 11.5 hours Summary: Negative					**Trial 2** - Harvest time: 12.0 hours Summary: Negative				
Medium					Medium				
	100	1	0.01	1.0		100	1	0.01	1.0
Mitomycin-C					Cyclophosphamide				
0.25	100	22	0.22	20.0	15	100	23	0.23	19.0
1.00	50	17	0.34	26.0	50	50	61	1.22	70.0
t-Butyl alcohol					*t*-Butyl alcohol				
1,600	100	5	0.05	5.0	1,600	100	4	0.04	4.0
3,000	100	3	0.03	3.0	3,000	100	2	0.02	2.0
4,000	100	0	0.00	0.0	4,000	100	2	0.02	2.0
5,000	100	5	0.05	5.0	5,000	13[c]	1	0.08	8.0
				P=0.360					P=0.334

* Positive ($P \leq 0.05$)
[a] Study performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented in Galloway *et al*. (1987). Abs = aberrations.
[b] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose
[c] Due to severe toxicity, only 13 cells were scored at this concentration.

TABLE D5
Induction of Micronuclei in Polychromatic Bone Marrow Cells of Male Rats Treated with *t*-Butyl Alcohol by Intraperitoneal Injection[a]

Dose	Number of Rats	Micronucleated PCEs/1,000 PCEs
Phosphate-Buffered Saline[b]		
0	5	1.70 ± 0.49
Cyclophosphamide[c]		
25	5	29.20 ± 3.76
t-Butyl Alcohol		
39.060	5	1.00 ± 0.35
78.125	4	1.00 ± 0.42
156.25		1.20 ± 0.41
312.5		0.90 ± 0.29
625	5	1.80 ± 0.25
1,250[d]	0	
		P=0.222[e]

[a] Study performed at Integrated Laboratory Systems, Inc. PCE = polychromatic erythrocyte.
[b] Solvent control
[c] Positive control
[d] All animals died at this dose level
[e] Significance tested by a one-tailed trend test

TABLE D6
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with *t*-Butyl Alcohol in Drinking Water for 13 Weeks[a]

	Dose (ppm)	Micronucleated NCEs[b] (%)	Micronucleated PCEs[b] (%)	Number of Mice
Male				
Urethane[c]	2,000	1.95 ± 0.07	1.79 ± 0.07	3
	0	0.09 ± 0.01	0.86 ± 0.14	8
	3,000	0.10 ± 0.01	1.03 ± 0.07	10
	5,000	0.07 ± 0.01	0.92 ± 0.16	10
	10,000	0.09 ± 0.02	0.68 ± 0.08	9
	20,000	0.08 ± 0.01	0.87 ± 0.10	9
	40,000	0.06 ± 0.03	0.52 ± 0.25	3
Female				
	0	0.06 ± 0.01	0.88 ± 0.13	9
	3,000	0.04 ± 0.01	0.68 ± 0.10	8
	5,000	0.05 ± 0.01	0.77 ± 0.16	10
	10,000	0.05 ± 0.01	0.88 ± 0.09	10
	20,000	0.07 ± 0.01	0.94 ± 0.08	9
	40,000	0.07 ± 0.01	0.81 ± 0.18	5

[a] Study performed at SRI, International. The detailed protocol is presented in MacGregor *et al*. (1990).
[b] Data are presented as mean ± standard error. NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte. Ten thousand NCEs and 2,000 PCEs were scored per animal. Results were not significant by a one-tailed trend test.
[c] Positive control; three male mice were dosed separately and were not part of the NTP toxicity study.