National Toxicology Program
Toxicity Report Series
Number 41

NTP Technical Report on the Toxicity Studies of

1,1,1-Trichlorethane

(CAS No. 76-55-6)

Administered in Microcapsules in Feed to F344/N Rats and B6C3F$_1$ Mice

August 2000

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

FOREWORD

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

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

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). Other information about NTP studies is available at the NTP's World Wide Web site: http//ntp-server.niehs.nih.gov.

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August 2000

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**U.S. Department of Health and Human Services
Public Health Service
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PEER REVIEW

The draft report on the toxicity studies of 1,1,1-trichloroethane 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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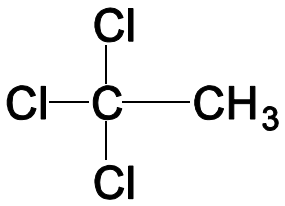
ABSTRACT



1,1,1-TRICHLOROETHANE

CAS No. 71-55-6

Chemical Formula: $C_2H_3Cl_3$ Molecular Weight: 133.40

Synonyms: Chloroethene; methylchloroform; methyl trichloromethane; α-trichloroethane; 1,1,1-TCE

1,1,1-Trichloroethane is a widely used solvent in industry and in household products such as cleaning agents, wallpaper and carpet glues, carpets, spray and solid insecticides, and rodenticides. 1,1,1-Trichloroethane was studied because of its widespread use in industry and in the home and the potential for human exposure. Groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice were given 5,000, 10,000, 20,000, 40,000, or 80,000 ppm microencapsulated 1,1,1,-trichloroethane in feed for 13 weeks. Groups of 10 male and 10 female rats and mice served as untreated controls and received feed without microcapsules; additional groups of 10 male and 10 female rats and mice served as vehicle controls and received feed with empty microcapsules. Animals were evaluated for clinical pathology (rats only), reproductive system effects, and histopathology. Genetic toxicity studies were conducted in *Salmonella typhimurium*, L5178Y mouse lymphoma cells, and cultured Chinese hamster ovary cells. In addition, peripheral blood slides from the mice in the 13-week study were analyzed for frequency of micronucleated erythrocytes.

All rats survived to the end of the study. The final mean body weights of exposed rats were within 10% of those of the untreated and vehicle controls. Feed consumption by exposed groups of male and female rats was similar to that by the control groups, suggesting that the diet was palatable to the animals. Based on average feed consumption values, male rats ingested approximately 300, 600, 1,200, 2,400, or 4,800 mg 1,1,1-trichloroethane/kg body weight per day, and females received 300, 650, 1,250, 2,500, or 5,000 mg/kg per day. In general, changes in clinical pathology parameters were minor, sporadic, and inconsistent between

males and females; these differences were not considered to be treatment related or biologically significant. The liver weights of female rats administered 80,000 ppm were significantly less than those of the untreated and vehicle controls. Male rats exposed to 10,000 ppm or greater had a spectrum of nonneoplastic kidney lesions consistent with hyaline droplet nephropathy. No treatment-related gross or microscopic lesions were observed in female rats.

There were no exposure-related deaths in mice. Based on average feed consumption values, male mice ingested approximately 850, 1,770, 3,500, 7,370, or 15,000 mg/kg per day, and female mice received 1,340, 2,820, 5,600, 11,125, or 23,000 mg/kg per day. Even though feed consumption by exposed groups was slightly greater than that by the controls, the mean body weights of male and female mice administered 20,000 ppm or greater were significantly less than those of the untreated and vehicle controls. The heart, kidney, and lung weights of the vehicle control male mice were significantly greater than those of the untreated controls. There were no biologically significant differences in organ weights between exposed and control mice. No gross or microscopic lesions in male or female mice were attributed to chemical exposure.

Epididymal spermatozoal concentrations of male rats and mice given 80,000 ppm were significantly less than those of the vehicle controls.

1,1,1-Trichloroethane was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without S9 metabolic activation. In the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells, 1,1,1-trichloroethane gave a negative response in one test (with and without S9) and an equivocal response in a second test (in the presence of S9). Results of a sister chromatid exchange test in cultured Chinese hamster ovary cells were considered to be equivocal due to an unrepeated questionable response obtained in the presence of S9 in a single trial; without S9, results were negative. 1,1,1-Trichloroethane induced chromosomal aberrations in cultured Chinese hamster ovary cells in the absence of S9; with S9, the increase in aberrations noted in a single trial was not significant. A small increase in the frequency of micronucleated normochromatic erythrocytes was noted in peripheral blood slides from male mice administered 1,1,1-trichloroethane in feed for 13 weeks; the results were determined to be equivocal, while the female peripheral blood micronucleus test results were negative.

In conclusion, 1,1,1-trichloroethane induced nonneoplastic lesions consistent with hyaline droplet nephropathy in male rats. Exposure to 1,1,1-trichloroethane caused decreases in liver weights in female rats and decreases in mean body weights of male and female mice. The no-observed-adverse-effect level (NOAEL) was estimated to be 10,000 ppm for male and female rats and mice.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

1,1,1-Trichloroethane is a colorless liquid with a boiling point of 74.1° C, a melting point of -32.5° C, and a density of 1.3376 at 20° C. 1,1,1-Trichloroethane is insoluble in water but is soluble in most organic solvents (*Merck Index*, 1989). 1,1,1-Trichloroethane is nonflammable, but decomposes at ambient temperatures in the presence of water and metals, liberating hydrochloric acid. It can also be oxidized at high temperatures to yield phosgene.

PRODUCTION, USE, AND HUMAN EXPOSURE

1,1,1-Trichloroethane is used as an industrial solvent, especially for the cold cleaning of metals, in dry cleaning, and in vapor degreasing, as an intermediate in the production of vinylidene chloride, and as an ingredient in cosmetics, aerosols, and adhesives (21 CFR, § 121.2520; 40 CFR, § 180.1001; Aviado, 1977; *Chemical and Engineering News*, 1979; IARC, 1979; *Kirk-Othmer*, 1979; *Chemical Marketing Reporter*, 1986). 1,1,1-Trichloroethane is widely used in household products such as liquid detergents, wallpaper and carpet glues, carpets, spray and solid insecticides, chlorine bleaches, scouring powders, and rodenticides (Wallace *et al.*, 1987).

Technical and solvent grades of 1,1,1-trichloroethane available in the United States vary only in the amount of stabilizer added to prevent metal corrosion (IARC, 1979; *Kirk-Othmer*, 1981). About 694.3 million pounds of 1,1,1-trichloroethane were produced in the United States during 1987 (USITC, 1989), and during 1985, 20 million pounds were imported (HSDB, 1988). However, production of 1,1,1-trichloroethane will be gradually phased out under the 1990 Amendments to the Clean Air Act (U.S.C., 42)

The potential for exposure of the general population to 1,1,1-trichloroethane is great because of its widespread use in industry and in the home. The primary route of exposure is by inhalation, although exposure may also occur topically or orally. 1,1,1-Trichloroethane has been identified as one of the chemicals most commonly found at hazardous waste sites that are on the National Priorities List (244 of 1,177 sites) (VIEW ATSDR, 1989). Comprehensive reviews of the health effects of 1,1,1-trichloroethane have been published by the Agency for Toxic Substances and Disease Registry (ATSDR, 1995) and the German Chemical Society (GDCh, 1994).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The absorption, disposition, metabolism, and excretion of 1,1,1-trichloroethane have been studied in humans and rodents; reviews of these studies have been published (ATSDR, 1995). 1,1,1-Trichloroethane is well absorbed by the lungs, reaching peak blood concentrations and approaching steady state within an hour after exposure begins. 1,1,1-Trichloroethane is fat soluble and is distributed primarily in the adipose and brain tissues, with lesser amounts in the liver and kidney (You and Dallas, 1998). Approximately 90% of the absorbed dose, regardless of exposure route, is rapidly excreted by the lungs as the parent compound. Little 1,1,1-trichloroethane is metabolized in humans, rats, or mice. In dogs, the half-life of 1,1,1-trichloroethane in blood was 4 to 12 minutes (Katagiri *et al.*, 1997). Urinary metabolites include trichloroethanol and, to a lesser extent, trichloroacetic acid; small amounts are converted to carbon dioxide and excreted by the lungs. Metabolism of 1,1,1-trichloroethane to acetylene by hepatic cytochrome P450 in rats has been demonstrated (Dürk, *et al.*, 1992).

TOXICITY

Experimental Animals

Median lethal concentrations (LC_{50}s) for 1,1,1-trichloroethane from inhalation studies of varying exposure periods have been estimated to be from 10,300 to 38,000 ppm (Adams *et al.*, 1950; Bonnet *et al.*, 1980; Clark and Tinston, 1982) for Sprague-Dawley, Alderly-Clark, and Wistar rats and from 3,911 to 18,358 ppm for CD-1 mice (Horiguchi and Horiguchi, 1971; Woolverton and Balster, 1981; Moser *et al.*, 1985). The variability in estimating the LC_{50}s is due to the difference in the length of the exposure period used for each study. The oral LD_{50} estimates for 1,1,1-trichloroethane obtained from gavage studies were 12,300 mg/kg for male rats, 10,300 mg/kg for female rats, and 11,240 mg/kg for male and female mice (Torkelson *et al.*, 1958).

Upon cessation of continuous inhalation of 1,1,1-trichloroethane (500 to 4,000 ppm), mice exhibited a withdrawal syndrome characterized by handling-induced convulsions and increased susceptibility to pentylenetetrazol-induced convulsions (Evans and Balster, 1993). The results of the study demonstrated that 1,1,1-trichloroethane has the ability to produce physical dependence similar to that induced by central nervous system depressant drugs. Bowen *et al.* (1996) reported that in a functional battery test study inhaled 1,1,1-trichloroethane induced changes in posture, decreased arousal, disturbed gait, decreased forelimb grip strength, increased landing foot splay, and impaired psychomotor coordination in male CFW albino mice. However, F344 rats exposed to 1,1,1-trichloroethane at up to 2,000 ppm, 6 hours per day, 5 days per week

for 13 weeks exhibited no neurotoxic effects as evaluated by a functional observational battery of tests (Mattsson *et al.*, 1993).

Rats exposed to 1,1,1-trichloroethane intragastrically at 5 mmole/kg had higher serum and perfusate concentrations of glutamic pyruvic transaminase, sorbitol dehydrogenase, and glutamate dehydrogenase than controls (Xia and Yu, 1992). Measurement of the relative total free radical concentration in the liver was also higher than in controls, additionally suggesting hepatotoxicity.

Humans

1,1,1-Trichloroethane is a solvent with a high potential for inhalation abuse, and death after inhalation of 1,1,1-trichloroethane has been reported (Winek *et al.*, 1997). Chronic exposure can produce damage to the liver, kidneys, and brain (Cohen and Frank, 1994; Flanagan and Ives, 1994). Anttila *et al.* (1995) reported that workers exposed to 1,1,1-trichloroethane had an increased risk of multiple myeloma and cancer of the nervous system.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

No dose-dependent effects on fertility, gestation, viability, or lactation were observed in male or female ICR Swiss mice exposed to 1,1,1,-trichloroethane in drinking water (containing up to 1% Emulphor EL-620) at up to 5,833 ppm (Lane *et al.*, 1982). Additionally, pup survival and weight gain were not adversely affected. In a study by George *et al.* (1989), no adverse effects were observed on reproductive parameters of male and female CD rats exposed to 3, 10, or 30 ppm 1,1,1-trichloroethane in drinking water (dispersed in Tween 80), and no developmental effects were observed in the offspring of these animals. In contrast, Dapson *et al.* (1984) reported cardiac abnormalities in offspring of Sprague-Dawley rats exposed to 10 ppm 1,1,1-trichloroethane in drinking water (also dispersed in Tween 80).

In inhalation studies, no developmental toxicity effects were observed in pregnant rats and mice exposed to 875 ppm 1,1,1-trichloroethane during gestation (Leong *et al.*, 1975). Fetotoxic effects were observed when pregnant rats were exposed to 2,100 ppm 1,1,1-trichloroethane (York *et al.*, 1982). Jones *et al.* (1996) reported no differences on pregnancy outcome when CD-1 mice were exposed to 2,000 ppm 1,1,1-trichloroethane during gestational days 12 through 17. However, the pups gained less weight, exhibited delays in developmental landmarks and acquisition of the righting reflex, performed poorer on tests of motor

coordination, and exhibited delays in negative geotaxis compared to controls. Similar results were observed when pregnant mice were exposed to 8,000 ppm 1,1,1-trichloroethane for 60 minutes, three times per day, during gestational days 12 through 17.

Humans

No information on the reproductive effects of 1,1,1-trichloroethane in humans was found in the literature.

CARCINOGENICITY

Maltoni *et al.* (1986) reported that Sprague-Dawley rats exposed to 500 mg/kg 1,1,1-trichloroethane in olive oil by gavage daily for 104 weeks developed an increase in the incidence of leukemia, but the results were considered inconclusive.

Quast *et al.* (1988) reported that Fischer rats and $B6C3F_1$ mice exposed to 1,1,1-trichloroethane at concentrations up to 1,500 ppm by inhalation for 2 years had no increases in neoplasm incidences compared to chamber controls. The National Cancer Institute also reported that rats administered 750 or 1,500 mg 1,1,1-trichloroethane per kilogram body weight by gavage and mice administered up to 6,000 mg/kg had no increased incidences of neoplasms, though these studies were considered inadequate due to poor survival and a short (78-week) duration of exposure (NCI, 1977).

GENETIC TOXICITY

Extensive mutagenicity testing of 1,1,1-trichloroethane was performed as part of the International Collaborative Program for the Evaluation of Short-Term Tests for Carcinogens (de Serres and Ashby, 1981). The summary reports from this collaborative program concluded that 1,1,1-trichloroethane was nonmutagenic in a variety of *in vitro* and *in vivo* test systems. A summary of these results is described below, along with results of tests that were performed independently from this collaborative effort. 1,1,1-Trichloroethane has been tested in numerous bacterial DNA repair and mutation assays, particularly the *Salmonella typhimurium* mutation assay, and results were generally negative (Brooks and Dean, 1981; MacDonald, 1981; Richold and Jones, 1981; Rowland and Severn, 1981; Trueman, 1981; Haworth *et al.*, 1983; Zeiger *et al.*, 1987). However, a few positive responses have been reported in *S. typhimurium* strains TA100 and TA1535, which mutate via base substitution (Nestmann *et al.*, 1980, 1984; Gocke *et al.*, 1981). These positive results were from assays in which bacteria were exposed to 1,1,1-trichloroethane and its vapors in the sealed atmosphere of a desiccator,

rather than in the standard plate incorporation or liquid preincubation treatment protocols that do not control for volatility. Therefore, it appears that 1,1,1-trichloroethane is mutagenic in *S. typhimurium* at high vapor concentrations when tested with a protocol appropriate for a volatile chemical (Nestmann *et al.*, 1984).

1,1,1-Trichloroethane was not mutagenic in tests with *Saccharomyces cerevisiae* that assayed for: mitotic gene conversion (Sharp and Parry, 1981; Zimmermann and Scheel, 1981); mitotic crossing-over and growth inhibition due to DNA damage (Kassinova *et al.*, 1981); mitotic aneuploidy (Parry and Sharp, 1981); or induction of gene reversion (Mehta and von Borstel, 1981). However, the studies were not conducted in a sealed atmosphere. Results from a single assay for induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* were negative (Gocke *et al.*, 1981).

In mammalian *in vitro* test systems, 1,1,1-trichloroethane was reported to be genotoxic in some assays. Equivocal results were reported from an assay for induction of trifluorothymidine resistance in mouse lymphoma cells treated with 1,1,1-trichloroethane in the presence of S9 due to erratic mutagenic activity observed among several experiments (Myhr and Caspary, 1988). Negative results, with and without S9, were reported for 1,1,1-trichloroethane in this same assay using doses as great or greater than were used in the equivocal test (Mitchell *et al.*, 1988). Equivocal results were also reported with 1,1,1-trichloroethane in an assay for induction of sister chromatid exchanges in cultured Chinese hamster ovary cells, with and without S9 (Galloway *et al.*, 1987). Chromosomal aberrations were induced by 1,1,1-trichloroethane in cultured Chinese hamster ovary cells treated in the absence of S9 activation (Galloway *et al.*, 1987); with S9, no increase in chromosomal aberrations was observed. Tests for induction of unscheduled (repair-type) DNA synthesis in rat hepatocytes (Althaus *et al.*, 1982) and human HeLa cells (Martin and McDermid, 1981) treated with 1,1,1-trichloroethane gave negative results.

Negative results were obtained *in vivo* with 1,1,1-trichloroethane in tests for induction of micronucleated erythrocytes (Gocke *et al.*, 1981; Salamone *et al.*, 1981; Tsuchimoto and Matter, 1981) and germ cell dominant lethal mutations (Lane *et al.*, 1982) in mice.

In conclusion, 1,1,1-trichloroethane has been tested in a variety of *in vitro* and *in vivo* assays for genotoxicity. Results from most bacterial mutation tests were negative, but positive responses were obtained in some assays that conducted exposures in closed systems that retained 1,1,1-trichloroethane vapors. *In vitro* mammalian cell assays were limited, but gave an indication of the ability of 1,1,1-trichloroethane to induce chromosomal damage in cultured Chinese hamster ovary cells. All results from *in vivo* tests for chromosomal damage in mice were negative.

STUDY RATIONALE AND DESIGN

In their review of the published 1,1,1-trichloroethane study data, the ATSDR identified the need for data from intermediate-duration oral exposure studies to provide information that would help determine the no-observed-adverse-effect levels and lowest-observed-adverse-effect levels for systemic, neurological, reproductive, and developmental effects. There are populations surrounding hazardous waste sites that might be exposed to 1,1,1-trichloroethane for more than a brief time, and data from these studies would help to estimate human risk of repeated oral exposure. To help fill the data gap on the effects of intermediate-duration oral exposure, the NTP conducted several short-term studies under an interagency agreement with the ATSDR.

This report describes the results of 13-week toxicity studies conducted using microencapsulated 1,1,1-trichloroethane mixed with feed and given to male and female F344/N rats and $B6C3F_1$ mice. 1,1,1-Trichloroethane was microencapsulated to avoid loss of chemical due to volatilization during dosed feed preparation and to avoid toxicity that might occur when a chemical is given in a bolus dose (e.g., gavage administration in corn oil). The NTP developmental toxicity studies have been reported elsewhere (George *et al.*, 1989).

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 1,1,1-TRICHLOROETHANE

1,1,1-Trichloroethane (lot TA 821004-1) was manufactured by Dow Chemical Company (Midland, MI). Initial purity and identity analyses were performed by Midwest Research Institute (MRI; Kansas City, MO). The microencapsulation of the chemical was performed by MRI. Microcapsule shells were composed of 80% food-grade modified corn starch and 20% sucrose (reagent grade).

The chemical, a clear, colorless liquid, was identified as 1,1,1-trichloroethane by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with literature spectra (*Sadtler Standard Spectra*). The density and boiling point were also consistent with literature references (*Merck Index*, 1983). The results of elemental analyses for carbon, hydrogen, and chlorine agreed with theoretical values. Karl Fischer water analysis indicated 0.03% $\pm$ 0.01% water. Free acid titration indicated less than 0.001 mEq acid/g sample. Gas chromatography by two systems with flame ionization detection indicated a major peak and no impurities with areas greater than 0.1% relative to the major peak. Cumulative data indicated a purity of greater than 99%.

Accelerated stability studies performed by MRI using gas chromatography with a flame ionization detector indicated that 1,1,1-trichloroethane was stable as a bulk chemical for at least 2 weeks when stored protected from light under a nitrogen headspace at temperatures up to 60° C.

Following microencapsulation of the chemical and prior to shipment to the study laboratory, the compound was tested by MRI for conformance to specifications. A profile conducted with U.S. standard sieves revealed that placebo and 1,1,1-trichloroethane-loaded capsules were within particle size specifications, and microscopic examination revealed no unusual characteristics. Chemical load was analyzed by gas chromatography with flame ionization detection; analyses revealed that the mean 1,1,1-trichloroethane load in the microcapsules was 50.8% $\pm$ 0.3%. Based upon analyses of samples from seven bottles of microcapsules, loads varied by no more than 0.8%. Microcapsules exposed to simulated study conditions (50% relative humidity at 25° C) in open dishes showed no measurable loss of chemical, measured by weight, after 28 days. Microcapsules that had been subjected to seven freeze-thaw cycles prior to an open-dish study retained 99.1% of their initial chemical load after 28 days. Microcapsules stored in a sealed bottle at 5° C for 28 days retained 97.6% by weight of

their chemical load. Results of gas chromatography profiles comparing impurities in the neat chemical and in microencapsulated 1,1,1-trichloroethane indicated no additional impurities resulting from encapsulation. Results of gas chromatography with electron capture analysis of the placebo capsules (used in preparing feed for vehicle controls in the present studies) indicated no detectable levels of 1,1,1-trichloroethane. The overall results indicated that microencapsulated 1,1,1-trichloroethane and the placebo capsules met all specifications. The bulk chemical was stored at 15° to 25° C, protected from light. The study laboratory monitored the stability of the microencapsulated chemical; no differences in the chemical load were detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

A premix of microencapsulated 1,1,1-trichloroethane or placebo microcapsules and Zeigler NIH-07 Open Formula mash or meal (Zeigler Brothers, Inc., Gardners, PA) was prepared for each dose formulation; additional portions of feed were added, and the premix was stirred with a spatula after each addition. For the final preparation, the premix and additional feed were layered in a twin-shell blender and blended for 20 minutes. The blend was removed from the blender and mixed manually, then returned to the blender and mixed for an additional 10 minutes.

Homogeneity and stability studies of the dosed feed mixture containing 2.5 mg 1,1,1-trichloroethane per gram of feed were performed at MRI with gas chromatography with a flame ionization detector. Homogeneity was confirmed. Stability studies showed that feed mixtures exhibited losses under a variety of storage conditions. Samples stored at room temperature in sealed containers lost 1.9%, 5.3%, and 7.3% after storage for 7, 14, and 21 days, respectively. A loss of 8.9% was observed in a dose formulation exposed to air, light, and 50% relative humidity at 26° C for 1 day, with no additional losses over the following 6 days. Throughout the studies, dose formulations were stored in sealed containers at room temperature for no longer than 3 weeks.

The study laboratory periodically analyzed the dose formulations with gas chromatography. All dose formulations administered were within 10% of theoretical concentrations. Analyses of animal room samples indicated increases in the concentrations of one rat and all mouse dose formulations, possibly due to selective feeding by the animals on feed rather than on microcapsules. However, some rat dose formulations had losses in concentration that were greater than expected, perhaps due to moisture in the feed hoppers.

13-WEEK STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, rats and mice were 4 to 5 weeks old. Animals were quarantined for 12 to 14 days; rats and mice were 6 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Serum samples were taken from five male and five female rats and mice before the studies began, from five male and five female rats on day 23, and from five male and five female mice at the end of the studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b); all results were negative.

The doses for the 13-week studies were based on the results of preliminary studies. Groups of 10 male and 10 female rats and mice were fed diets containing 5,000, 10,000, 20,000, 40,000, or 80,000 ppm microencapsulated 1,1,1-trichloroethane. Additional groups of 10 male and 10 female rats and mice received untreated feed (untreated controls) or feed containing placebo microcapsules (vehicle controls). Rats were housed five per cage and mice were housed individually. NIH-07 Open Formula mash (rats) or meal (mice) and water were available *ad libitum*. Clinical findings were recorded weekly; animals were weighed initially and weekly thereafter. Details of the study design and animal maintenance are summarized in Table 1.

Blood for clinical pathology evaluations was collected from additional groups of 10 male and 10 female rats (exposed to the same concentrations of 1,1,1-trichloroethane as the core study rats) on days 3 and 23 and from all core study rats at the end of the 13-week study. Animals were anesthetized with carbon dioxide, and blood was drawn from the retroorbital sinus. Samples for hematology analyses were placed in tubes containing potassium EDTA; samples for clinical chemistry were placed in tubes without anticoagulant. Samples for clinical chemistry analyses were allowed to clot at room temperature and centrifuged, and the serum was removed.

Hematologic determinations were made on a Sysmex TOA E-2500 hematology analyzer (TOA Medical Electronics, Inc., Ltd., Kobe, Japan) with reagents obtained from Baxter Scientific Products (McGaw Park, IL). The parameters evaluated are listed in Table 1. Leukocyte differential counts and morphologic evaluation of blood cells were determined by light microscopy from blood smears stained with Wright-Giemsa. Smears made from blood samples stained with new methylene blue N were examined microscopically for quantitative determination of reticulocytes.

Clinical chemistry parameters were measured on a Roche Cobas FARA automated centrifugal analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). The parameters that were measured are listed in Table 1. Reagents for analyses of sorbitol dehydrogenase and bile acids were obtained from Sigma Chemical Company (St. Louis, MO); other reagents were obtained from the equipment manufacturer.

Urine samples were collected from groups of five randomly selected male rats in the vehicle control, 5,000, 20,000, and 80,000 ppm groups on days 28 and 84 of the study. Rats were placed in metabolism cages for approximately 24 hours; the volume of collected samples was measured, and the samples were stored on ice. Samples were mixed with deionized water, and urine creatinine, trichloroacetic acid, and free and total trichloroethanol concentrations were measured with gas chromatography; hydrochloric acid was added to the samples to aid the extraction of trichloroacetic acid.

Vaginal cytology and sperm motility evaluations were performed on rats and mice at the end of the 13-week core studies. Ten male and female rats and mice in the vehicle control, 20,000, 40,000, and 80,000 ppm groups were evaluated, and the parameters measured are listed in Table 1. Methods were those outlined in the National Toxicology Program's Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and Mice (NTP, 1987). For 12 days prior to sacrifice, the vaginal vaults of 10 females per group were lavaged, and the aspirated vaginal 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 identify estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Sperm motility was evaluated at necropsy. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body 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 on two slides for five microscopic fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in phosphate-buffered saline solution. Caudae were finely minced, and the tissue was incubated and then heat fixed. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lungs, right testis, and thymus of all core study animals were weighed. Organs and tissues were examined for gross lesions and fixed

in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on untreated controls, vehicle controls, and core study rats and mice exposed to 80,000 ppm. Tissues and organs routinely examined are listed in Table 1.

Upon completion of the laboratory pathologist's histopathologic 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).

TABLE 1
Experimental Design and Materials and Methods in the 13-Week Feed Studies of 1,1,1-Trichloroethane

Study Laboratory	TSI Mason Research Institute (Worcester, MA)
Strain and Species	F344/N rats $B6C3F_1$ mice
Animal Source	Taconic Farms (Germantown, NY)
Time Held Before Studies	Rats: 14 (males) or 12 (females) days Mice: 13 (males) or 14 (females) days
Average Age When Studies Began	Rats: 6 weeks Mice: 6 to 7 weeks
Date of First Exposure	Rats: 16 (females) or 18 (males) April 1990 Mice: 9 (males) or 10 (females) May 1990
Duration of Exposure	13 weeks (7 days/week)
Date of Last Exposure and Necropsy	Rats: 16 (females) or 18 (males) July 1990 Mice: 8 (males) or 9 (females) August 1990
Average Age at Necropsy	Rats: 19 weeks Mice: 19 (males) or 20 (females) weeks
Size of Study Groups	10 males and 10 females
Method of Distribution	Animals were distributed randomly into groups of approximately equal initial mean body weight.
Animals per Cage	Rats: 5 Mice: 1
Method of Animal Identification	Tail tattoo
Diet	NIH-07 Open Formula mash (rats) or meal (mice) (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly
Water	Tap water (Worcester municipal supply) in glass water bottles (Allentown Caging Inc., Allentown, NJ), available *ad libitum*
Cages	Polycarbonate (Lab Products, Inc., Rochelle Park, NJ), changed twice per week
Bedding	Sani Chips heat-treated hardwood chips (P.J. Murphy Products Corp., Montville, NJ), changed twice per week
Cage Filters	Nonwoven fiber (Snow Filtration, Cincinnati, OH), changed once every 2 weeks
Racks	Stainless steel (Lab Products, Inc., Rochelle Park, NJ), changed once every 2 weeks
Animal Room Environment	Temperature: 20.0° to 23.3° C (rats); 19.4° to 24.4° C (mice) Relative humidity: 45% to 64% (rats); 42% to 66% (mice) Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Exposure Concentrations	0, 5,000, 10,000, 20,000, 40,000, or 80,000 ppm, microencapsulated in feed, available *ad libitum*

TABLE 1
Experimental Design and Materials and Methods in the 13-Week Feed Studies of 1,1,1-Trichloroethane

Type and Frequency of Observation	Animals were observed twice daily and were weighed initially and weekly thereafter. Clinical findings were recorded weekly. Feed consumption was recorded by cage twice weekly (at 3- to 4-day intervals).
Method of Sacrifice	Carbon dioxide asphyxiation
Necropsy	Necropsies were performed on all core study animals. The heart, right kidney, liver, lungs, right testis, and thymus were weighed.
Clinical Pathology	On days 3 and 23 of exposure, blood was collected from the retroorbital sinus of rats in the clinical pathology groups under anesthesia with carbon dioxide. Additional samples were taken similarly from core study rats at the end of the study. Urine samples were collected on days 28 and 84 of the study from male rats in the vehicle control, 5,000, 20,000, and 80,000 ppm clinical pathology groups. ***Hematology:*** Hematocrit (automated and manual); hemoglobin; erythrocyte, reticulocyte, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count; and leukocyte count and differentials ***Clinical chemistry:*** Blood urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids ***Urinalysis and metabolites:*** Urine volume, urine creatinine, trichloroacetic acid, and free and total trichloroethanol
Histopathology	Complete histopathologic examinations were performed on untreated controls, vehicle controls, and core study rats and mice exposed to 80,000 ppm. In addition to gross lesions and tissue masses, the following tissues were evaluated microscopically: adrenal gland, bone and marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular stomach), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. Additionally, the kidney of rats in all exposed groups in the core study were examined.
Sperm Motility and Vaginal Cytology	Sperm motility and vaginal cytology evaluations were performed on core study rats and mice in the vehicle control, 20,000, 40,000, and 80,000 ppm groups. Male rats and mice were evaluated for necropsy body and reproductive tissue weights, epididymal spermatozoal data, and spermatogenesis. Female rats and mice were evaluated for necropsy body weight, estrous cycle length, and percentage of estrous cycle spent in various stages.

STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

The incidences of lesions as presented in Appendix A are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. The Fisher exact test, a procedure based on the overall proportion of affected animals, was used to determine significance (Gart *et al.*, 1979).

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 historically have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoa data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations.

QUALITY ASSURANCE METHODS

The animal studies of 1,1,1-trichloroethane were performed in compliance with United States Food and Drug Administration Good Laboratory Practices regulations (21 CFR, Part 58). The Quality Assurance Unit of TSI Mason Research Institute performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Haworth *et al*. (1983) and Zeiger *et al*. (1987). 1,1,1-Trichloroethane was sent to each of the testing laboratories as a coded aliquot from Radian Corporation (Austin, TX) and 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 of at least five doses of 1,1,1-trichloroethane. The high dose was limited by toxicity in the tests performed at SRI International; in the tests performed at Case Western Reserve University, toxicity was not apparent, and 10,000 μg/plate was selected as the high dose. All trials were repeated; because the data are published, only one trial per strain and S9 condition is presented in this report.

A positive response in the *Salmonella typhimurium* assay 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 Mitchell *et al*. (1988) and Myhr and Caspary (1988). 1,1,1-Trichloroethane was supplied to the two testing laboratories as a coded aliquot by Radian Corporation. The high dose of 1,1,1-trichloroethane was determined by solubility and toxicity. L5178Y mouse lymphoma cells were maintained at 37° C as suspension cultures in supplemented Fischer's medium; normal cycling time was approximately 10 hours. To reduce the number of spontaneously occurring cells resistant to trifluorothymidine (TFT), subcultures were exposed once to medium containing thymidine, hypoxanthine, methotrexate, and glycine for 1 day; to medium containing thymidine, hypoxanthine, and glycine 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 levels within an experiment were replicated, including concurrent positive and solvent controls. Treated cultures contained 6×10^6 cells in 10 mL medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with 1,1,1-trichloroethane continued for 4 hours, at which time the medium plus 1,1,1-trichloroethane 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 TFT for selection of TFT-resistant cells, and cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% carbon dioxide for 10 to 12 days. The assays were initially performed without S9; because a clearly positive response was not obtained, the experiments were repeated using freshly prepared S9 from the livers of either Aroclor 1254-induced male Sprague-Dawley rats or Syrian hamsters.

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 both trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for 1,1,1-trichloroethane to be considered capable of inducing TFT resistance. A single significant response led to a "questionable" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

Chinese Hamster Ovary Cell Cytogenetics Protocols

Testing was performed as reported by Galloway *et al*. (1987). 1,1,1-Trichloroethane 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 three doses of 1,1,1-trichloroethane; the high dose was limited by toxicity in most trials. In the absence of toxicity, 5 mg/mL was selected as the high dose. A single flask per dose was used.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with 1,1,1-trichloroethane in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing 1,1,1-trichloroethane 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 1,1,1-trichloroethane, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no 1,1,1-trichloroethane. 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 concentration.

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 ($P < 0.005$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with 1,1,1-trichloroethane for 12 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 1,1,1-trichloroethane and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 12 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. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test.

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 ten or more aberrations).

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 in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.*, 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 13-week toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange. Slides were

scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per exposure 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 NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposure group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

RESULTS

RATS

All rats survived to the end of the study (Table 2). The final mean body weight and body weight gain of males given 10,000 ppm were significantly greater than those of the untreated controls (Table 2 and Figure 1). The final mean body weight and body weight gain of females in the 20,000 ppm group were significantly less than those of the untreated controls. The final mean body weights and body weight gains of males in the 40,000 and 80,000 ppm groups and the final mean body weight of females in the 80,000 ppm group were significantly less than those of the vehicle controls. The final mean body weight and body weight gain of vehicle control male rats were significantly greater than those of the untreated controls. Feed consumption by exposed rats was generally similar to that by the control groups. Exposure concentrations of 5,000, 10,000, 20,000, 40,000, and 80,000 ppm resulted in average daily doses of approximately 300, 600, 1,200, 2,400, and 4,800 mg 1,1,1-trichloroethane/kg body weight to male rats and 300, 650, 1,250, 2,500, and 5,000 mg/kg to female rats. However, feed consumption values and exposure concentrations were determined by the disappearance of feed from the feeder and may not accurately represent intake. There were no clinical findings related to chemical exposure.

The liver weights of females given 80,000 ppm were significantly less than those of the untreated and vehicle controls (Tables 3 and C1). Other organ weight differences were not considered to be related to chemical exposure.

TABLE 2
Survival, Body Weights, and Feed and Compound Consumption of Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Untreated Controls (%)	Average Feed Consumption[c] (g/kg/day)	Average Dose[c] (mg/kg/day)
Male							
Untreated Control	10/10	144 ± 3	321 ± 4	177 ± 3		58.8	
Vehicle Control	10/10	148 ± 3	340 ± 6*	192 ± 6*		58.5	
5,000	10/10	146 ± 3	332 ± 4	186 ± 4	98	57.9	290
10,000	10/10	148 ± 3	344 ± 4**	196 ± 4**	101	60.7	600
20,000	10/10	149 ± 3	336 ± 4	187 ± 5	99	59.4	1,200
40,000	10/10	146 ± 3	322 ± 4▲	175 ± 3▲	95	59.4	2,400
80,000	10/10	147 ± 3	307 ± 6▲▲	161 ± 6*▲▲	90	60.2	4,800
Female							
Untreated Control	10/10	107 ± 1	188 ± 2	81 ± 2		62.6	
Vehicle Control	10/10	108 ± 2	189 ± 3	81 ± 3		62.8	
5,000	10/10	108 ± 2	194 ± 3	86 ± 3	103	62.5	310
10,000	10/10	108 ± 2	189 ± 3	81 ± 2	100	64.3	650
20,000	10/10	106 ± 2	178 ± 3*	72 ± 2*	94	62.9	1,250
40,000	10/10	105 ± 2	188 ± 2	83 ± 2	99	62.2	2,500
80,000	10/10	106 ± 2	181 ± 2▲	75 ± 2	96	62.6	5,000

* Significantly different ($P \leq 0.05$) from the untreated control group by Dunnett's test
** $P \leq 0.01$
▲ Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' 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.
[c] Average of individual consumption values for weeks 1 through 13 for animals in the core study

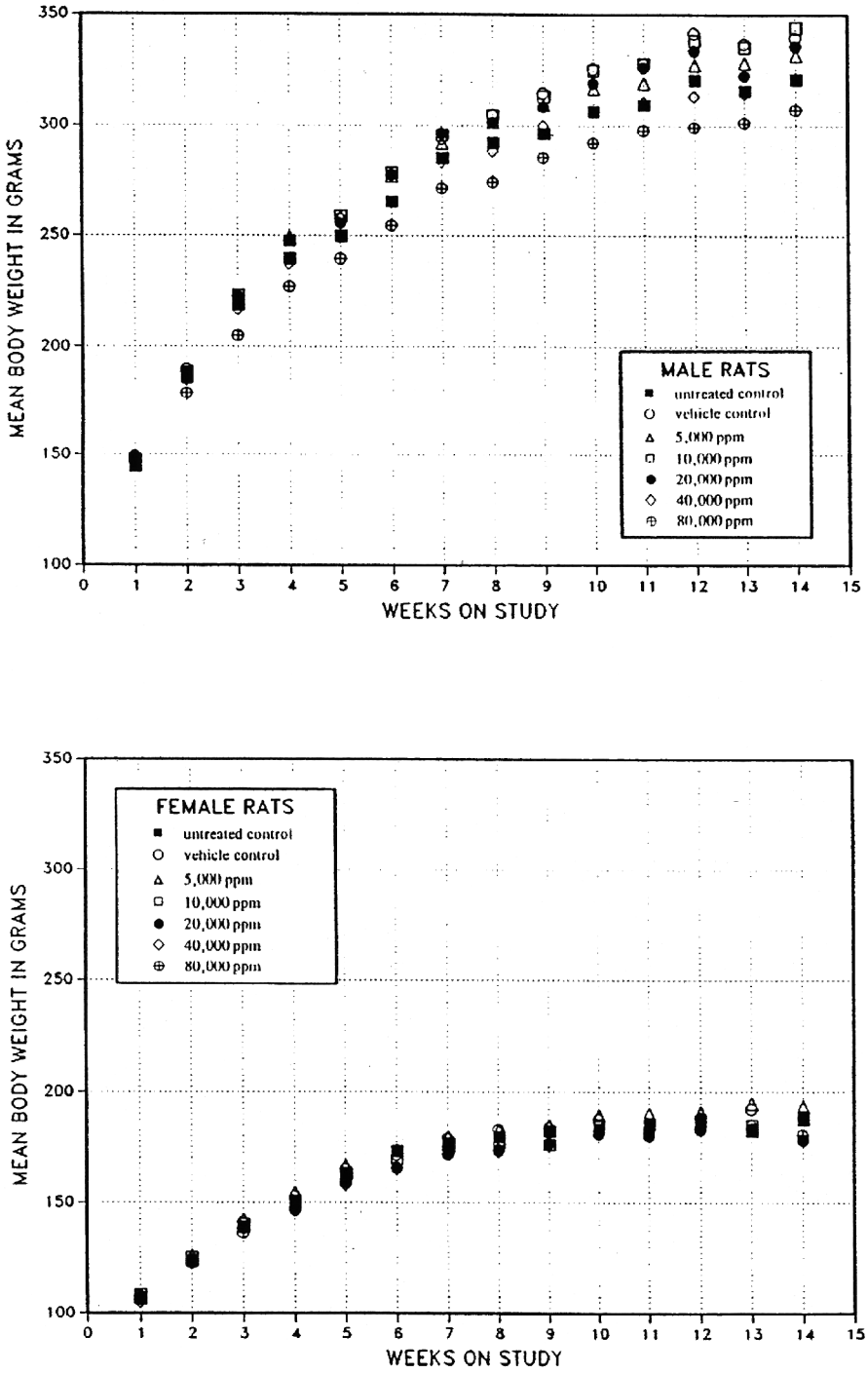


FIGURE 1
Body Weights for Male and Female Rats Exposed to 1,1,1-Trichloroethane in Feed for 13 Weeks

TABLE 3
Liver Weights and Liver-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
n	10	10	10	10	10	10	10
Male							
Necropsy body wt	310 ± 4	326 ± 7*	321 ± 3	332 ± 4**	325 ± 4*	310 ± 4▲	296 ± 6▲▲
Liver							
Absolute	12.059 ± 0.272	13.050 ± 0.404	13.181 ± 0.262	14.206 ± 0.262**	13.294 ± 0.446	12.469 ± 0.419	11.370 ± 0.391▲▲
Relative	38.94 ± 0.61	39.94 ± 0.59	41.11 ± 0.67	42.81 ± 0.76*	40.87 ± 1.11	40.14 ± 1.02	38.41 ± 0.89
Female							
Necropsy body wt	184 ± 2	184 ± 3	188 ± 3	184 ± 3	173 ± 3	181 ± 2	175 ± 2*▲
Liver							
Absolute	6.090 ± 0.179	6.187 ± 0.156	6.513 ± 0.308	6.333 ± 0.125	5.744 ± 0.180	6.067 ± 0.188	5.151 ± 0.086**▲▲
Relative	33.14 ± 0.96	33.69 ± 0.65	34.67 ± 1.71	34.41 ± 0.43	33.19 ± 0.65	33.46 ± 0.89	29.53 ± 0.38*▲▲

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

Hematology and clinical chemistry data for rats are listed in Table B1. The only apparent treatment-related effects were observed in the hematology parameters and alkaline phosphatase activities. In general, a minimal erythrocytosis, evidenced by increased hematocrit values, hemoglobin concentrations, and erythrocyte counts occurred in 10,000 ppm or greater males and females on days 3 and 23. These findings would be consistent with a minimal relative erythrocytosis related to hemoconcentration. Additionally, alkaline phosphatase activities were minimally decreased in exposed groups at various time points.

Urinary volume measurements and creatinine concentrations of exposed groups of male rats were similar to those of the vehicle controls (Table B2). Trichloroacetic acid and total trichloroethanol concentrations of 5,000, 20,000, and 80,000 ppm males were significantly greater than those of the vehicle controls on days 28 and 84, as were free trichloroethanol concentrations on day 28 (Figure 2 and Table B2).

A spectrum of nonneoplastic kidney lesions was observed in male rats exposed to 20,000 ppm or greater (Tables 4 and A1). The individual components of nephropathy increased in incidence and/or severity with exposure concentration; these components included renal tubule hyaline degeneration, regeneration, cast formation, and chronic interstitial inflammation. Hyaline degeneration was characterized by an accumulation of hyaline droplets within the cytoplasm of the epithelial cells lining the proximal convoluted tubules. These droplets were of greater size and number than those in untreated or vehicle control males and often had an angular shape, in contrast to the spherical droplets seen in the controls. Tubular regeneration consisted of small foci of cortical tubules with increased basophilia and nuclear/cytoplasmic ratio. Tubular casts were characterized by the presence of granular material within the lumen of tubules at the cortico-medullary junction. No gross or microscopic lesions in female rats were attributed to 1,1,1-trichloroethane exposure.

The epididymal spermatozoal concentration of males in the 80,000 ppm group was significantly less (~10%) than that of the vehicle controls (Table D1); no other male reproductive parameters were affected. Vaginal cytology parameters of exposed groups of females were similar to those of the vehicle controls (Table D2).

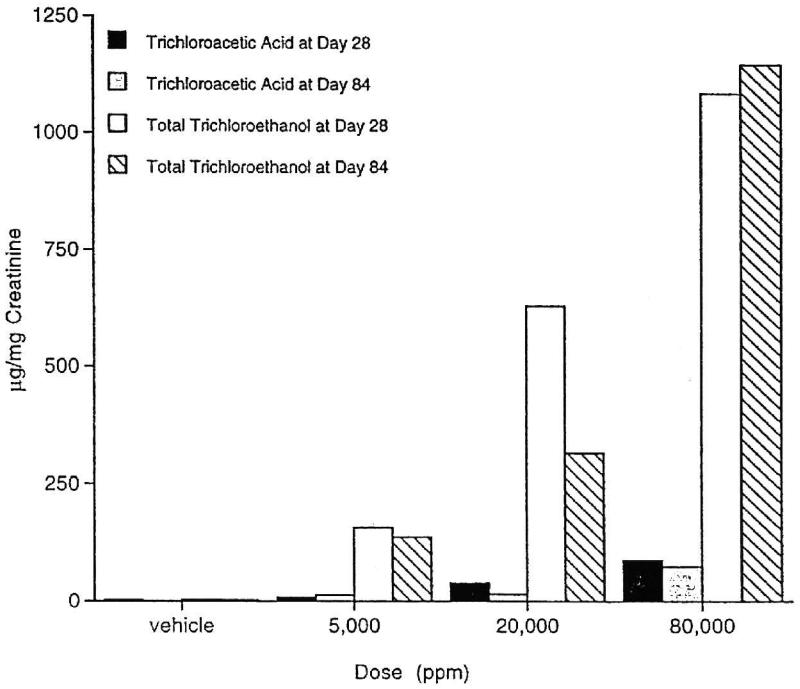


FIGURE 2
Urinary Metabolites in Male Rats Exposed to 1,1,1-Trichloroethane in Feed for 13 Weeks

TABLE 4
Incidences of Nonneoplastic Lesions of the Kidney in Male Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Number Examined Microscopically	10	10	10	10	10	10	10
Inflammation, Chronic[a]	0	0	0	2 (1.0)[b]	7**▲▲ (1.0)	10**▲▲ (1.1)	10**▲▲ (1.2)
Renal Tubule, Casts	0	0	0	0	1 (1.0)	5*▲ (1.0)	10**▲▲ (2.0)
Renal Tubule, Degeneration, Hyaline	0	0	0	0	10**▲▲ (1.2)	10**▲▲ (1.1)	10**▲▲ (2.0)
Renal Tubule, Regeneration	10 (1.0)	10 (1.2)	10 (1.1)	10 (1.5)	10 (1.8)	10 (1.8)	10 (2.2)

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

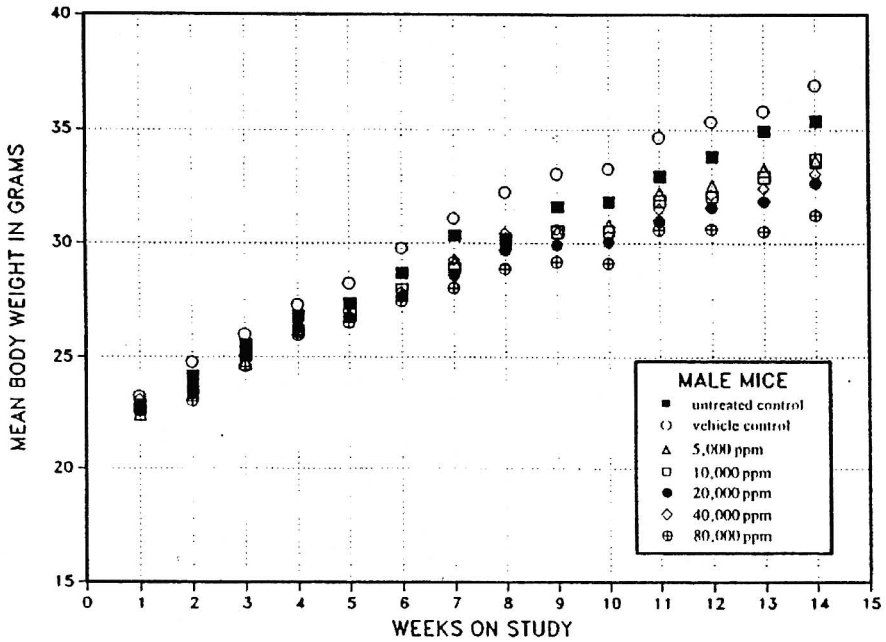
MICE

There were no exposure-related deaths (Table 5). The final mean body weights and body weight gains of male and female mice given 20,000 ppm or greater were significantly less than those of the untreated and vehicle controls (Table 5 and Figure 3). The mean body weight gains of males in the 5,000 and 10,000 ppm groups were also significantly less than those of the untreated and vehicle control groups. The final mean body weights of males exposed to 5,000 or 10,000 ppm and the mean body weight gain of females in the 10,000 ppm group were significantly less than those of the vehicle controls. Feed consumption by exposed mice was generally slightly greater than that by the untreated and vehicle controls. Exposure concentrations of 5,000, 10,000, 20,000, 40,000, and 80,000 ppm resulted in average daily doses of approximately 850, 1,770, 3,500, 7,370, and 15,000 mg/kg to male mice and 1,340, 2,820, 5,600, 11,125, and 23,000 mg/kg to female mice. However, feed consumption values and exposure concentrations were determined by the disappearance of feed from the feeder and may not accurately represent intake. There were no clinical findings related to chemical exposure.

TABLE 5
Survival, Body Weights, and Feed and Compound Consumption of Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Untreated Controls (%)	Average Feed Consumption[c] (g/kg/day)	Average Dose[c] (mg/kg/day)
Male							
Untreated Control	9/10[d]	22.6 ± 0.4	35.4 ± 0.8	12.8 ± 0.5		160	—
Vehicle Control	10/10	23.3 ± 0.4	36.9 ± 0.7	13.7 ± 0.5		156	—
5,000	10/10	22.4 ± 0.3	33.6 ± 0.7▲▲	11.2 ± 0.5*▲▲	91	170	850
10,000	10/10	22.9 ± 0.2	33.7 ± 0.6▲▲	10.8 ± 0.5**▲▲	91	177	1,750
20,000	10/10	22.8 ± 0.4	32.7 ± 0.5**▲▲	9.9 ± 0.4**▲▲	88	177	3,500
40,000	10/10	23.1 ± 0.4	33.1 ± 0.5**▲▲	10.0 ± 0.3**▲▲	90	184	7,370
80,000	10/10	22.6 ± 0.2	31.3 ± 0.4**▲▲	8.7 ± 0.3**▲▲	85	187	15,000
Female							
Untreated Control	10/10	18.7 ± 0.3	28.8 ± 0.9	10.1 ± 0.8		250	—
Vehicle Control	10/10	18.0 ± 0.3	29.3 ± 0.8	11.2 ± 0.8		261	—
5,000	10/10	18.8 ± 0.1	28.4 ± 0.6	9.6 ± 0.7	97	268	1,340
10,000	10/10	18.6 ± 0.2	27.2 ± 0.8	8.7 ± 0.6▲▲	93	282	2,820
20,000	10/10	18.5 ± 0.2	26.0 ± 0.8**▲▲	7.5 ± 0.7**▲▲	89	280	5,600
40,000	10/10	18.6 ± 0.1	25.8 ± 0.7**▲▲	7.2 ± 0.6**▲▲	88	278	11,125
80,000	8/10[e]	18.4 ± 0.2	24.5 ± 0.5**▲▲	6.2 ± 0.5**▲▲	84	287	22,900

* Significantly different ($P \leq 0.05$) from the untreated control group by Williams' test
** $P \leq 0.01$
▲▲ Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[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] Average of individual consumption values for weeks 1 through 13 for animals in the core study
[d] Week of death: 4 (missing)
[e] Week of death: 12 (one missing, one accidental death)



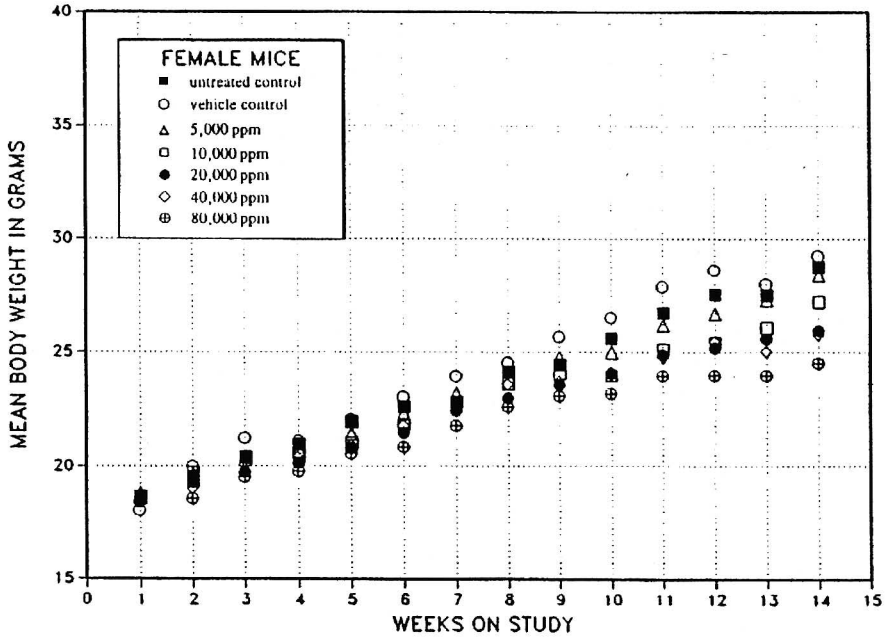


FIGURE 3
Body Weights for Male and Female Mice Exposed to 1,1,1-Trichloroethane in Feed for 13 Weeks

Relative right kidney and liver weights of exposed males were generally significantly greater than those of the untreated controls (Tables 6 and C2). Females exposed to 20,000 ppm or greater had significantly greater relative right kidney weights than the untreated controls. Absolute heart weights of males in all exposed groups and relative heart weights of males in the 40,000 and 80,000 ppm groups were significantly less than those of the vehicle controls. The heart, right kidney, and lung weights of males in the vehicle control group were significantly greater than those of the untreated controls. Differences in organ weights between exposed and control mice were considered to be secondary to body weight changes and were not considered to be biologically significant.

No gross or microscopic lesions that could be attributed to exposure to microencapsulated 1,1,1-trichloroethane were observed in male or female mice.

Males in the 80,000 ppm group had a significantly lower (~20%) epididymal spermatozoal concentration than the vehicle controls (Table D3); no other male reproductive parameters were affected. Vaginal cytology parameters of exposed groups of females were similar to those of the vehicle controls (Table D4).

TABLE 6
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Male							
n	9	10	10	10	10	10	10
Necropsy body wt	31.7 ± 0.7	32.7 ± 1.3	30.5 ± 0.7	30.6 ± 0.9	30.4 ± 0.7	30.0 ± 0.5▲	28.0 ± 0.4**▲▲
Heart							
Absolute	0.161 ± 0.007	0.194 ± 0.007**	0.167 ± 0.006▲▲	0.174 ± 0.008▲▲	0.168 ± 0.003▲▲	0.159 ± 0.005▲▲	0.150 ± 0.004▲▲
Relative	5.08 ± 0.18	5.97 ± 0.19**	5.46 ± 0.15	5.69 ± 0.24	5.54 ± 0.10	5.30 ± 0.12▲▲	5.36 ± 0.16▲▲
R. Kidney							
Absolute	0.289 ± 0.013	0.333 ± 0.008**	0.310 ± 0.009▲	0.312 ± 0.006▲	0.310 ± 0.006▲	0.312 ± 0.008▲	0.296 ± 0.006▲▲
Relative	9.11 ± 0.33	10.32 ± 0.43*	10.17 ± 0.20*	10.29 ± 0.40*	10.23 ± 0.34*	10.40 ± 0.26**	10.58 ± 0.20**
Liver							
Absolute	1.676 ± 0.045	1.855 ± 0.046*	1.717 ± 0.039	1.752 ± 0.044	1.747 ± 0.040	1.828 ± 0.061	1.646 ± 0.040▲▲
Relative	52.90 ± 1.00	57.39 ± 2.15	56.35 ± 0.68	57.79 ± 2.49*	57.46 ± 0.86*	60.85 ± 1.54**	58.71 ± 1.36**
Lung							
Absolute	0.213 ± 0.012	0.251 ± 0.008*	0.237 ± 0.011[b]	0.236 ± 0.016	0.234 ± 0.013	0.237 ± 0.015	0.224 ± 0.014
Relative	6.69 ± 0.26	7.80 ± 0.40*	7.82 ± 0.32[b]	7.74 ± 0.50	7.71 ± 0.47	7.88 ± 0.49	8.02 ± 0.53
Female							
n	10	10	10	10	10	10	8
Necropsy body wt	26.8 ± 0.9	28.1 ± 0.8	26.8 ± 0.6	26.8 ± 0.8	25.3 ± 0.8▲▲	25.0 ± 0.7▲▲	24.1 ± 0.5*▲▲
Heart							
Absolute	0.139 ± 0.005	0.138 ± 0.003	0.156 ± 0.010	0.150 ± 0.012	0.129 ± 0.006	0.129 ± 0.005	0.125 ± 0.007
Relative	5.22 ± 0.23	4.94 ± 0.14	5.82 ± 0.36	5.61 ± 0.41	5.12 ± 0.17	5.19 ± 0.20	5.18 ± 0.18
R. Kidney							
Absolute	0.192 ± 0.005	0.215 ± 0.005**	0.205 ± 0.003	0.205 ± 0.004	0.201 ± 0.004▲	0.193 ± 0.004▲▲	0.195 ± 0.002▲▲
Relative	7.19 ± 0.17	7.67 ± 0.17	7.70 ± 0.24	7.67 ± 0.18	8.00 ± 0.24*	7.75 ± 0.24*	8.11 ± 0.16**

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

GENETIC TOXICOLOGY

1,1,1-Trichloroethane (up to 10,000 μg/plate) was tested in four separate assays at two different laboratories for induction of mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, with and without induced hamster or rat liver S9; all tests were negative (Table E1; Haworth *et al.*, 1983; Zeiger *et al.*, 1987). The test protocol employed in these *Salmonella* assays did not control for volatility and, therefore, actual exposure levels may have been lower than indicated. In the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells (Table E2), 1,1,1-trichloroethane gave a negative response at one laboratory, with and without S9 (Mitchell *et al.*, 1988), and an equivocal response at a second laboratory in the presence of induced S9 (Myhr and Caspary, 1988). In the study performed at SRI International, a positive response was obtained in the first trial conducted with S9, but a second trial showed no evidence of induced mutagenicity within the same dose range tested in the first trial, and the results were concluded to be negative. In the study performed at Litton Bionetics, Inc., 1,1,1-trichloroethane was clearly nonmutagenic in the absence of S9, but with S9, an erratic response was obtained among four trials, leading to the overall call of equivocal. The first two trials were clearly positive. A small increase in revertant colonies was observed at the highest concentration level (0.5 μg/mL) in the third trial. This response was invalidated due to the presence of a precipitate at this concentration, however, and the trial was concluded to be negative. The fourth trial in the presence of S9 showed a complete absence of response at all dose levels. In cytogenetic tests with cultured Chinese hamster ovary cells, 1,1,1-trichloroethane gave an equivocal response in the sister chromatid exchange test (Table E3; Galloway *et al.*, 1987) and a positive response in the test for induction of chromosomal aberrations (Table E4; Galloway *et al.*, 1987). In the sister chromatid exchange test without S9, the first trial was concluded to be equivocal because, although a significant increase in sister chromatid exchanges was observed at the highest dose tested (500 μg/mL), the trend was not significant (P=0.031). The positive response observed in the first trial was not reproduced in a second trial. With S9, the results of a single trial were considered to be equivocal because the trend was highly significant (P=0.003) even though a significant increase in sister chromatid exchanges was not observed at any of the individual dose points. In the chromosomal aberrations test, positive responses were obtained at two of the three doses tested in the absence of S9, and this trial was positive despite the lack of a positive trend. 1,1,1-Trichloroethane (up to 5,000 μg/mL) did not induce a significant increase in chromosomal aberrations in the presence of S9. Peripheral blood slides from male and female mice were evaluated for frequency of micronucleated normochromatic erythrocytes. The results in male mice were equivocal based on an increase in normochromatic erythrocytes that correlated with exposure and produced a positive trend test (P=0.013), but the treated values were not significantly increased when compared to the controls. In female mice, the frequency of normochromatic erythrocytes at 2,000 ppm was elevated when compared to the controls, but because the increase was small and there was no dose response, the results in female mice were negative.

In conclusion, 1,1,1-trichloroethane was not mutagenic in *Salmonella*, and the results from mammalian cell mutagenicity assays were equivocal. It is possible that the volatility of 1,1,1-trichloroethane was a factor in these results. There was evidence of chromosomal damage in cultured Chinese hamster ovary cells exposed to 1,1,1-trichloroethane, but results from the micronucleus test in mice did not give clear evidence of induced chromosomal effects *in vivo*.

DISCUSSION

There were no early deaths of untreated or vehicle control rats or mice. The final mean body weight and body weight gain of vehicle control male rats were significantly greater than those of the untreated controls. The vehicle microcapsules probably increased the caloric density of the feed; average feed consumption was similar between the groups. No effect on mean body weight gains was observed in female vehicle control rats or male or female vehicle control mice. No histopathologic changes were observed in vehicle control rats or mice compared to the untreated controls. The NTP has conducted many studies with microencapsulated chemicals and found no adverse effects (Yuan *et al.*, 1992). Thus, microencapsulation appears to be an excellent tool for administering volatile chemicals in dosed feed studies.

All rats exposed to 1,1,1-trichloroethane survived to the end of the study. Final mean body weights and body weight gains of males exposed to 40,000 or 80,000 ppm and the final mean body weight of females in the 80,000 ppm group were significantly less than those of the vehicle controls; however, the final mean body weights were within 10% of those of the vehicle and untreated controls. Similarly, there were no treatment-related deaths in the mice. Final mean body weights and body weight gains of all groups of exposed males and female mice administered 20,000 ppm or greater were significantly less than those of the vehicle controls, as was the mean body weight gain of females in the 10,000 ppm group. Higher concentrations and prolonged administration of 1,1,1-trichloroethane appear to depress body weight gains in rats and mice. This body weight effect was not due to reduced feed consumption; feed consumption by exposed rats and mice was similar to or greater than that by the control groups. 1,1,1-Trichloroethane administered in corn oil by gavage to Osborne-Mendel rats at 0, 750, or 1,500 mg/kg per day and to $B6C3F_1$ mice at time-weighted average doses of 2,807 or 5,615 mg/kg per day for 78 weeks caused moderate decreases in the mean body weight gain of male rats and of male and female mice (NCI, 1977). Increases in erythrocyte parameters and decreases in blood alkaline phosphatase activity were observed in rats exposed to high concentrations of 1,1,1-trichloroethane. However, the changes were minimal and may be due to physiological processes unrelated to the effects of the 1,1,1-trichloroethane.

Liver weights were decreased in female rats in the 80,000 ppm group compared to the control groups; this effect was not observed in exposed male rats. In spite of the reduction in body weight gains of exposed groups of male and female mice, no relative organ weight differences compared to the vehicle controls were observed other than increased relative heart weights in males in the 40,000 and 80,000 ppm groups. Thus, body weight

reductions did not affect any organ specifically. In another NTP study in which male F344/N rats were administered 0.62 or 1.24 mmol 1,1,1-trichloroethane per kilogram body weight by gavage for 3 weeks, no body weight or organ weight effects were observed (NTP, 1996).

The only histopathologic changes observed in rats or mice fed the microencapsulated 1,1,1-trichloroethane occurred in the kidneys of male rats. The spectrum of kidney lesions observed in male rats exposed to 20,000 ppm or greater is considered to be related to exposure to 1,1,1-trichloroethane. This complex of lesions is consistent with hyaline droplet nephropathy, which results from the accumulation of α2u-globulin in the renal tubules; however, α2u-globulin concentrations were not determined in this study. Similar kidney lesions were not observed in female rats or male or female mice; neither α2u-globulin nor hyaline droplets were found in female rats or male or female mice. Many chemicals are known to induce hyaline droplets or α2u-globulin in renal proximal tubules of male rats. This induction leads to necrosis of the tubule epithelium, regenerative tubule cell proliferation, development of intraluminal granular casts from sloughed cell debris, tubule hyperplasia and, often, a low incidence of renal tubule neoplasms (USEPA, 1991). 1,1,1-Trichloroethane appears to belong to this category of chemicals which induce nephropathy in male rats. The nephropathy is reversible after exposure to the chemical ends. These reversible kidney lesions are not considered applicable to human risk assessment.

Most of the administered 1,1,1-trichloroethane is excreted unchanged via the lungs and the remainder is distributed to adipose tissue, liver, and kidney and is metabolized to trichloroethanol and trichloroacetic acid and excreted via urine (ATSDR, 1995). The rat data indicated that the amounts of trichloroacetic acid and trichloroethanol excreted were dose dependent.

Epididymal spermatozoal concentrations of male rats and mice given 80,000 ppm were significantly less than those of the vehicle controls; this effect has been correlated with reduced fertility (Chapin *et al.*, 1997). There were no treatment-related effects on vaginal cytology parameters of female rats or mice. The selective reduction in epididymal sperm count, without a concomitant reduction in testicular spermatid measures, suggests an effect of increased urinary (or ejaculatory) sperm loss in rats and mice. While the inhibition of body weight gain may have produced reproductive system changes in mice fed a restricted diet, it was ineffective in Sprague-Dawley rats (Chapin *et al.*, 1993a). Additionally, other changes have been seen in feed-restricted mice that were not observed in this study (Chapin *et al.*, 1993b). In summary, it appears that this slight reduction in epididymal sperm counts is a real treatment effect, probably affecting the epididymis.

In conclusion, 1,1,1-trichloroethane induced nonneoplastic lesions consistent with hyaline droplet nephropathy in male rats. Exposure to 1,1,1-trichloroethane caused decreases in liver weights in female rats and decreases in mean body weights of male and female mice. The no-observed-adverse-effect level (NOAEL) was estimated to be 10,000 ppm for male and female rats and mice.

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APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS AND MICE

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane A-2
TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane A-4
TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane A-6
TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane A-8

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Survivors							
Terminal sacrifice	10	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10	10
Alimentary System							
Intestine, large, cecum							(10)
Dilatation							1 (10%)
Liver	(10)	(10)	(2)	(2)			
Cytoplasmic alteration				1 (50%)			
Hepatodiaphragmatic nodule		1 (10%)		1 (50%)			
Mesentery					(1)		
Fat, hemorrhage					1 (100%)		
Pancreas	(10)	(10)					(10)
Acinus, atrophy	1 (10%)	1 (10%)					1 (10%)
Cardiovascular System							
Heart	(10)	(10)					(10)
Cardiomyopathy	9 (90%)	9 (90%)					10 (100%)
Endocrine System							
None							
General Body System							
None							
Genital System							
Preputial gland	(10)	(10)					(10)
Abscess							1 (10%)
Inflammation, acute		1 (10%)					
Hematopoietic System							
Spleen							(10)
Congestion							1 (10%)
Integumentary System							
None							
Musculoskeletal System							
None							

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Nervous System							
Brain	(10)	(10)					
Hemorrhage		1 (10%)					
Respiratory System							
Lung							(10)
Hemorrhage							1 (10%)
Special Senses System							
None							
Urinary System							
Kidney	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic				2 (20%)	7 (70%)	10 (100%)	10 (100%)
Cortex, mineralization	2 (20%)	2 (20%)					1 (10%)
Renal tubule, casts					1 (10%)	5 (50%)	10 (100%)
Renal tubule, degeneration, hyaline					10 (100%)	10 (100%)	10 (100%)
Renal tubule, regeneration	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Urinary bladder	(10)	(10)	(1)	(1)			
Calculus, gross observation		2 (20%)	1 (100%)	1 (100%)			
Calculus, microscopic observation only		1 (10%)	1 (100%)	1 (100%)			

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

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Survivors							
Terminal sacrifice	10	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10	10
Alimentary System							
Intestine large, cecum	(10)	(10)				(1)	
Dilatation						1 (100%)	
Liver	(10)	(10)	(1)		(2)		(10)
Hepatodiaphragmatic nodule			1 (100%)		1 (50%)		
Inflammation, chronic							1 (10%)
Necrosis							1 (10%)
Pancreas	(10)	(10)					
Acinus, atrophy		1 (10%)					
Cardiovascular System							
Heart	(10)	(10)					(10)
Cardiomyopathy	7 (70%)	6 (60%)					9 (90%)
Endocrine System							
None							
General Body System							
None							
Genital System							
Clitoral gland	(10)	(10)	(1)				
Dilatation		1 (10%)	1 (100%)				
Ovary	(10)	(10)					(10)
Cyst		1 (10%)					1 (10%)
Uterus	(10)	(10)	(1)			(3)	
Dilatation	1 (10%)		1 (100%)				
Hematopoietic System							
Lymph node				(1)			
Mediastinal, angiectasis				1 (100%)			
Lymph node, mandibular	(10)	(10)			(1)		
Hyperplasia, lymphoid					1 (100%)		
Spleen	(10)	(10)				(1)	
Congestion						1 (100%)	

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Integumentary System							
None							
Musculoskeletal System							
Skeletal muscle							(1)
Atrophy							1 (100%)
Nervous System							
None							
Respiratory System							
None							
Special Senses System							
None							
Urinary System							
Kidney	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic		3 (30%)	1 (10%)			2 (20%)	4 (40%)
Cortex, mineralization	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Renal tubule, regeneration	8 (80%)	7 (70%)	5 (50%)	6 (60%)	5 (50%)	9 (90%)	7 (70%)

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

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Survivors							
Terminal sacrifice	9	10	10	10	10	10	10
Missing	1						
Animals examined microscopically	9	10					10
Alimentary System							
Liver	(9)	(10)					(10)
Hematopoietic cell proliferation	1 (11%)	2 (20%)					6 (60%)
Necrosis		1 (10%)					
Cardiovascular System							
None							
Endocrine System							
None							
General Body System							
None							
Genital System							
None							
Hematopoietic System							
Spleen	(9)	(10)					(10)
Hematopoietic cell proliferation	8 (89%)	9 (90%)					10 (100%)
Integumentary System							
None							
Musculoskeletal System							
None							
Nervous System							
None							

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Respiratory System							
Lung	(9)	(10)					(10)
Hemorrhage	2 (22%)	4 (40%)					3 (30%)
Special Senses System							
Eye		(1)					
Lens, cataract		1 (100%)					
Urinary System							
Kidney	(9)	(10)					(10)
Inflammation, chronic		1 (10%)					1 (10%)

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Early deaths							
Accidental death							1
Survivors							
Terminal sacrifice	10	10	10	10	10	10	8
Missing							1
Animals examined microscopically	10	10					9
Alimentary System							
Liver	(10)	(10)					(9)
Hematopoietic cell proliferation	7 (70%)	7 (70%)					8 (89%)
Necrosis		1 (10%)					
Pigmentation							1 (11%)
Cardiovascular System							
None							
Endocrine System							
Adrenal cortex							(9)
Accessory adrenal cortical nodule							1 (11%)
General Body System							
None							
Genital System							
None							
Hematopoietic System							
Spleen	(10)	(10)					(9)
Hematopoietic cell proliferation	10 (100%)	10 (100%)					9 (100%)
Integumentary System							
None							
Musculoskeletal System							
None							
Nervous System							
None							

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Respiratory System							
Lung	(10)	(10)					(9)
Hemorrhage		2 (20%)					4 (44%)
Special Senses System							
None							
Urinary System							
Kidney							(9)
Renal tubule, casts							1 (11%)
Renal tubule, regeneration							1 (11%)

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

APPENDIX B
HEMATOLOGY, CLINICAL CHEMISTRY, URINALYSIS, AND URINARY METABOLITE RESULTS

TABLE B1 Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane B-2
TABLE B2 Urinalysis and Urinary Metabolite Data for Male Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane B-7

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Male							
Hematology							
n							
Day 3	10	10	10	10	10	10	10
Day 23	10	10	10	10	10	10	9
Week 13	10	10	10	10	10	10	10
Hematocrit (automated) (%)							
Day 3	43.4 ± 0.4	42.3 ± 0.6	43.5 ± 0.5	43.8 ± 0.2▲	43.7 ± 0.4▲	43.9 ± 0.6▲	45.4 ± 0.4**▲▲
Day 23	48.2 ± 0.4	47.8 ± 0.4	48.7 ± 0.2	49.0 ± 0.3	49.6 ± 0.6▲	49.0 ± 0.4	50.0 ± 0.4**▲▲
Week 13	47.3 ± 0.4	47.9 ± 0.5	47.0 ± 0.6	45.5 ± 0.5▲	45.9 ± 0.6	47.7 ± 0.6	47.0 ± 0.7
Hematocrit (manual) (%)							
Day 3	42.2 ± 0.4	41.3 ± 0.5	42.5 ± 0.5	42.6 ± 0.3▲	42.6 ± 0.3▲	42.5 ± 0.7	44.3 ± 0.5*▲▲
Day 23	46.5 ± 0.5	46.3 ± 0.4	46.6 ± 0.2	47.1 ± 0.3	47.6 ± 0.7	47.5 ± 0.5	48.4 ± 0.6*▲▲
Week 13	45.2 ± 0.4	46.2 ± 0.6*	45.4 ± 0.5	44.1 ± 0.5▲	44.2 ± 0.6▲	45.4 ± 0.4	45.1 ± 0.7
Hemoglobin (g/dL)							
Day 3	14.2 ± 0.1	13.8 ± 0.2	14.3 ± 0.1	14.3 ± 0.1	14.3 ± 0.1	14.3 ± 0.2	14.8 ± 0.1**▲▲
Day 23	16.1 ± 0.1	15.9 ± 0.2	16.1 ± 0.1	16.3 ± 0.1▲	16.4 ± 0.2	16.4 ± 0.2	16.6 ± 0.1*▲▲
Week 13	15.3 ± 0.1	15.4 ± 0.2	15.4 ± 0.2	14.8 ± 0.2	14.9 ± 0.2	15.3 ± 0.2	15.3 ± 0.2
Erythrocytes ($10^6/\mu L$)							
Day 3	7.20 ± 0.08	7.10 ± 0.09	7.23 ± 0.07	7.24 ± 0.06	7.23 ± 0.05	7.25 ± 0.09	7.54 ± 0.07*▲▲
Day 23	8.37 ± 0.05	8.28 ± 0.08	8.49 ± 0.07▲	8.59 ± 0.07▲▲	8.71 ± 0.10*▲▲	8.53 ± 0.08▲	8.68 ± 0.08*▲▲
Week 13	9.53 ± 0.11	9.67 ± 0.10	9.42 ± 0.12	9.21 ± 0.10	9.28 ± 0.10	9.66 ± 0.13	9.39 ± 0.12
Reticulocytes ($10^6/\mu L$)							
Day 3	0.25 ± 0.02	0.22 ± 0.02	0.30 ± 0.01▲▲	0.26 ± 0.02	0.24 ± 0.01	0.25 ± 0.01	0.26 ± 0.02
Day 23	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.17 ± 0.01
Week 13	0.20 ± 0.01	0.21 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.18 ± 0.01	0.22 ± 0.01	0.19 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)							
Day 3	0.09 ± 0.03	0.05 ± 0.02	0.07 ± 0.02	0.09 ± 0.03	0.05 ± 0.02	0.03 ± 0.02	0.02 ± 0.01
Day 23	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.03 ± 0.01	0.02 ± 0.02	0.01 ± 0.01
Week 13	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.04 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01
Mean cell volume (fL)							
Day 3	60.2 ± 0.3	59.6 ± 0.3	60.1 ± 0.4	60.6 ± 0.3	60.5 ± 0.3	60.5 ± 0.3	60.4 ± 0.3
Day 23	57.9 ± 0.3	57.9 ± 0.2	57.3 ± 0.3	57.2 ± 0.4	56.9 ± 0.4	57.4 ± 0.3	57.7 ± 0.2
Week 13	49.6 ± 0.3	49.7 ± 0.2	50.1 ± 0.3	49.5 ± 0.3	49.5 ± 0.2	49.4 ± 0.2	50.0 ± 0.3
Mean cell hemoglobin (pg)							
Day 3	19.7 ± 0.1	19.5 ± 0.1	19.8 ± 0.1▲	19.8 ± 0.1▲	19.7 ± 0.1	19.7 ± 0.1	19.6 ± 0.1
Day 23	19.3 ± 0.1	19.2 ± 0.1	18.9 ± 0.1	19.0 ± 0.1	18.9 ± 0.1	19.2 ± 0.1	19.1 ± 0.1
Week 13	16.0 ± 0.2	15.9 ± 0.1	16.4 ± 0.1▲	16.1 ± 0.1	16.1 ± 0.1	15.8 ± 0.1	16.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)							
Day 3	32.7 ± 0.1	32.7 ± 0.2	32.9 ± 0.2	32.7 ± 0.2	32.7 ± 0.2	32.6 ± 0.2	32.6 ± 0.1
Day 23	33.5 ± 0.2	33.2 ± 0.1	33.0 ± 0.2	33.4 ± 0.2	33.1 ± 0.2	33.4 ± 0.2	33.2 ± 0.1
Week 13	32.3 ± 0.2	32.1 ± 0.2	32.7 ± 0.1	32.5 ± 0.1	32.6 ± 0.1	32.0 ± 0.3	32.5 ± 0.2
Platelets ($10^3/\mu L$)							
Day 3	1,080 ± 15	1,120 ± 11*	1,157 ± 23	1,155 ± 24	1,141 ± 22	1,078 ± 25	1,175 ± 28*
Day 23	866 ± 21	929 ± 15	944 ± 14	914 ± 23	977 ± 26**	929 ± 23	920 ± 26
Week 13	757 ± 10	745 ± 13	736 ± 20	699 ± 17	762 ± 13	756 ± 16	754 ± 11[b]

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Male (continued)							
Hematology (continued)							
n							
Day 3	10	10	10	10	10	10	10
Day 23	10	10	10	10	10	10	9
Week 13	10	10	10	10	10	10	10
Leukocytes ($10^3/\mu L$)							
Day 3	10.00 ± 0.31	9.95 ± 0.65	9.28 ± 0.31	10.12 ± 0.31	9.36 ± 0.26	9.54 ± 0.23	10.23 ± 0.20
Day 23	10.16 ± 0.23	10.23 ± 0.50	9.81 ± 0.27	10.14 ± 0.31	9.74 ± 0.21	10.37 ± 0.21	10.46 ± 0.35
Week 13	8.87 ± 0.39	9.53 ± 0.50	9.88 ± 0.49	9.60 ± 0.28	9.44 ± 0.45	10.07 ± 0.30	9.69 ± 0.56
Segmented neutrophils ($10^3/\mu L$)							
Day 3	1.37 ± 0.11	1.51 ± 0.19	1.48 ± 0.13	1.45 ± 0.14	1.21 ± 0.12	1.64 ± 0.15	1.61 ± 0.17
Day 23	1.47 ± 0.08	1.23 ± 0.16	1.22 ± 0.14	1.23 ± 0.13	1.14 ± 0.11	1.08 ± 0.10	1.10 ± 0.13
Week 13	1.13 ± 0.15	1.08 ± 0.13	1.39 ± 0.16	1.30 ± 0.18	0.96 ± 0.08	1.55 ± 0.21	1.54 ± 0.19
Lymphocytes ($10^3/\mu L$)							
Day 3	8.60 ± 0.25	8.40 ± 0.63	7.72 ± 0.27	8.59 ± 0.32	8.03 ± 0.21	7.82 ± 0.21	8.50 ± 0.24
Day 23	8.63 ± 0.22	8.91 ± 0.46	8.49 ± 0.29	8.84 ± 0.38	8.51 ± 0.28	9.11 ± 0.22	9.21 ± 0.27
Week 13	7.68 ± 0.28	8.37 ± 0.47	8.48 ± 0.43	8.25 ± 0.29	8.47 ± 0.47	8.47 ± 0.35	8.07 ± 0.43
Monocytes ($10^3/\mu L$)							
Day 3	0.02 ± 0.01	0.03 ± 0.02	0.07 ± 0.03	0.06 ± 0.03	0.11 ± 0.03*	0.08 ± 0.03	0.09 ± 0.02
Day 23	0.05 ± 0.02	0.07 ± 0.04	0.05 ± 0.02	0.03 ± 0.02	0.06 ± 0.02	0.11 ± 0.02	0.09 ± 0.07
Week 13	0.03 ± 0.02	0.05 ± 0.03	0.00 ± 0.00	0.05 ± 0.02	0.00 ± 0.00	0.01 ± 0.01	0.04 ± 0.02
Eosinophils ($10^3/\mu L$)							
Day 3	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Day 23	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.07 ± 0.02	0.06 ± 0.02
Week 13	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.04 ± 0.02
Clinical Chemistry							
n	10	10	10	10	10	10	10
Urea nitrogen (mg/dL)							
Day 3	20.1 ± 0.6	18.0 ± 0.5*	20.1 ± 0.8	19.0 ± 0.8	20.0 ± 0.5	17.8 ± 0.6*	17.6 ± 0.3**
Day 23	23.4 ± 0.5	23.1 ± 0.4	22.6 ± 0.5	23.7 ± 0.5	24.4 ± 1.1	23.3 ± 0.3	24.6 ± 0.6
Week 13	20.9 ± 0.7	21.2 ± 0.4	21.2 ± 0.4	21.3 ± 0.7	21.5 ± 0.4	20.7 ± 0.5	22.2 ± 0.5
Creatinine (mg/dL)							
Day 3	0.53 ± 0.02	0.54 ± 0.02	0.57 ± 0.03	0.53 ± 0.02	0.56 ± 0.03	0.58 ± 0.02	0.46 ± 0.02
Day 23	0.51 ± 0.02	0.51 ± 0.02	0.57 ± 0.02	0.57 ± 0.02	0.54 ± 0.02	0.57 ± 0.02	0.53 ± 0.03
Week 13	0.66 ± 0.02	0.67 ± 0.02	0.70 ± 0.02	0.68 ± 0.02	0.70 ± 0.02	0.67 ± 0.02	0.70 ± 0.02
Total protein (g/dL)							
Day 3	6.1 ± 0.0	6.0 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	6.0 ± 0.1
Day 23	6.5 ± 0.1	6.7 ± 0.0*	6.6 ± 0.1	6.8 ± 0.1*	6.9 ± 0.1*	6.7 ± 0.1	6.7 ± 0.1
Week 13	6.5 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.7 ± 0.1
Albumin (g/dL)							
Day 3	3.2 ± 0.1	3.2 ± 0.0	3.3 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.1
Day 23	3.5 ± 0.0	3.6 ± 0.0	3.6 ± 0.0	3.6 ± 0.0	3.7 ± 0.1	3.6 ± 0.1	3.5 ± 0.1
Week 13	3.7 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.7 ± 0.0	3.8 ± 0.1

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Male (continued)							
Clinical Chemistry (continued)							
n	10	10	10	10	10	10	10
Alanine aminotransferase (IU/L)							
Day 3	46 ± 1	41 ± 1*	41 ± 1	43 ± 1	44 ± 1	43 ± 1	43 ± 2
Day 23	49 ± 1	53 ± 3	49 ± 3	55 ± 4	48 ± 2	45 ± 1	48 ± 1
Week 13	48 ± 1	48 ± 2	46 ± 2	50 ± 1[b]	43 ± 1	46 ± 2	42 ± 1*
Alkaline phosphatase (IU/L)							
Day 3	674 ± 9	626 ± 11**	672 ± 13▲	665 ± 10	650 ± 13	653 ± 11	624 ± 14**
Day 23	465 ± 11	467 ± 7	476 ± 5	465 ± 7	458 ± 8	453 ± 7	437 ± 7*▲▲
Week 13	254 ± 6	239 ± 3*	237 ± 4*	233 ± 5*	234 ± 6*	220 ± 6**	232 ± 5**
Creatine kinase (IU/L)							
Day 3	414 ± 78	333 ± 30	382 ± 37[b]	366 ± 68	506 ± 66[c]	477 ± 50	311 ± 47
Day 23	233 ± 29	238 ± 32	365 ± 68	250 ± 40[b]	282 ± 37	279 ± 59	331 ± 74
Week 13	217 ± 25[b]	164 ± 26	259 ± 65	154 ± 18	205 ± 43	165 ± 21	214 ± 34
Sorbitol dehydrogenase (IU/L)							
Day 3	8 ± 1	8 ± 0	7 ± 0	7 ± 1	6 ± 0	6 ± 0▲	7 ± 0
Day 23	9 ± 0	10 ± 1	9 ± 1	9 ± 1	8 ± 0▲▲	9 ± 0▲	8 ± 0▲▲
Week 13	7 ± 0	7 ± 0	7 ± 0	7 ± 0[b]	6 ± 0	7 ± 0	6 ± 1
Bile acids (μmol/L)							
Day 3	28.0 ± 3.7	33.7 ± 3.8[b]	37.6 ± 4.5	38.6 ± 4.4	46.6 ± 4.4**	43.0 ± 4.0**	44.9 ± 3.2**
Day 23	34.1 ± 5.0	35.2 ± 4.3[b]	39.8 ± 5.1	36.0 ± 3.4	43.1 ± 4.5	41.7 ± 4.3	48.8 ± 4.7
Week 13	22.2 ± 3.2	22.0 ± 2.0	31.3 ± 5.2	22.2 ± 1.7	35.4 ± 2.8*▲▲	29.7 ± 4.3	31.4 ± 2.3
Female							
Hematology							
n	10	10	10	10	10	10	10
Hematocrit (automated) (%)							
Day 3	43.6 ± 0.4	43.6 ± 0.4	43.9 ± 0.6	44.0 ± 0.3	44.5 ± 0.5	44.8 ± 0.4	44.8 ± 0.6
Day 23	46.8 ± 0.5	46.4 ± 0.4	46.7 ± 0.3	47.2 ± 0.4	46.5 ± 0.5	46.7 ± 0.4	48.1 ± 0.3▲
Week 13	45.5 ± 0.3	45.5 ± 0.3	45.4 ± 0.3	45.8 ± 0.3	47.5 ± 0.8▲	47.3 ± 0.4**▲▲	49.3 ± 0.4**▲▲
Hematocrit (manual) (%)							
Day 3	41.4 ± 0.4	42.1 ± 0.4	42.3 ± 0.5	42.6 ± 0.6	42.7 ± 0.5	43.5 ± 0.4**	43.2 ± 0.5**
Day 23	46.0 ± 0.7	45.6 ± 0.5	45.4 ± 0.4	45.5 ± 0.4	45.6 ± 0.5	45.8 ± 0.4	46.9 ± 0.3
Week 13	43.5 ± 0.4	44.0 ± 0.5	43.8 ± 0.3	43.7 ± 0.3	45.7 ± 0.6**	45.4 ± 0.5**	47.0 ± 0.5**▲▲
Hemoglobin (g/dL)							
Day 3	14.4 ± 0.1	14.5 ± 0.2	14.6 ± 0.2	14.6 ± 0.1	14.9 ± 0.2	14.9 ± 0.1*	15.1 ± 0.2*▲
Day 23	16.0 ± 0.1	16.0 ± 0.1	16.0 ± 0.1	16.0 ± 0.1	15.9 ± 0.1	15.9 ± 0.1	16.4 ± 0.1
Week 13	15.3 ± 0.1	15.3 ± 0.1	15.4 ± 0.1	15.5 ± 0.1	15.9 ± 0.3	15.9 ± 0.2**▲	16.4 ± 0.2**▲▲
Erythrocytes (10^6/μL)							
Day 3	7.35 ± 0.09	7.36 ± 0.09	7.47 ± 0.11	7.50 ± 0.06	7.61 ± 0.11	7.61 ± 0.07▲	7.72 ± 0.11*▲
Day 23	8.07 ± 0.08	7.99 ± 0.05	8.12 ± 0.07	8.14 ± 0.07	8.22 ± 0.09	8.09 ± 0.08	8.45 ± 0.08*▲▲
Week 13	8.59 ± 0.11	8.48 ± 0.14	8.46 ± 0.14	8.52 ± 0.17	9.11 ± 0.19▲	8.79 ± 0.16	9.38 ± 0.08**▲▲

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Female (continued)							
Hematology (continued)							
n	10	10	10	10	10	10	10
Reticulocytes ($10^6/\mu L$)							
Day 3	0.23 ± 0.01	0.20 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.21 ± 0.01	0.24 ± 0.02	0.24 ± 0.01
Day 23	0.13 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Week 13	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.02	0.17 ± 0.01	0.19 ± 0.01	0.19 ± 0.02	0.19 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)							
Day 3	0.06 ± 0.02	0.10 ± 0.03	0.05 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.07 ± 0.03
Day 23	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01
Week 13	0.04 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.07 ± 0.03	0.02 ± 0.01	0.03 ± 0.02
Mean cell volume (fL)							
Day 3	59.4 ± 0.3	59.3 ± 0.5	58.8 ± 0.3	58.7 ± 0.3	58.4 ± 0.3*	58.8 ± 0.3	58.1 ± 0.2**
Day 23	58.1 ± 0.4	58.1 ± 0.4	57.6 ± 0.4	58.0 ± 0.2	56.7 ± 0.3*▲	57.8 ± 0.4	57.0 ± 0.3
Week 13	50.0 ± 2.4	53.8 ± 0.7	53.9 ± 0.7	54.0 ± 1.0	52.3 ± 0.7	53.9 ± 0.9	52.6 ± 0.3
Mean cell hemoglobin (pg)							
Day 3	19.6 ± 0.1	19.7 ± 0.1	19.6 ± 0.1	19.5 ± 0.1	19.6 ± 0.1	19.5 ± 0.1	19.5 ± 0.1
Day 23	19.8 ± 0.2	20.0 ± 0.1	19.7 ± 0.1	19.7 ± 0.1	19.4 ± 0.1*▲▲	19.7 ± 0.1▲▲	19.5 ± 0.1▲▲
Week 13	17.8 ± 0.2	18.1 ± 0.3	18.2 ± 0.3	18.2 ± 0.3	17.5 ± 0.3	18.1 ± 0.4	17.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)							
Day 3	33.0 ± 0.2	33.2 ± 0.3	33.3 ± 0.2	33.3 ± 0.2	33.5 ± 0.1	33.2 ± 0.2	33.6 ± 0.1
Day 23	34.2 ± 0.1	34.4 ± 0.3	34.3 ± 0.1	34.0 ± 0.1	34.2 ± 0.2	34.1 ± 0.2	34.2 ± 0.2
Week 13	33.6 ± 0.1	33.8 ± 0.2	33.9 ± 0.1	33.8 ± 0.2	33.5 ± 0.2	33.5 ± 0.1	33.4 ± 0.2
Platelets ($10^3/\mu L$)							
Day 3	1,193 ± 13	1,157 ± 25	1,161 ± 21	1,183 ± 28	1,167 ± 21	1,174 ± 34	1,185 ± 33
Day 23	833 ± 13	827 ± 26	871 ± 18	891 ± 18	874 ± 14	827 ± 24	869 ± 17
Week 13	759 ± 32	727 ± 53	792 ± 55	712 ± 47	807 ± 36	701 ± 52	779 ± 21
Leukocytes ($10^3/\mu L$)							
Day 3	8.80 ± 0.38	9.28 ± 0.40	9.00 ± 0.27	8.90 ± 0.36	10.09 ± 0.44	10.05 ± 0.36*	10.62 ± 0.38**▲
Day 23	9.77 ± 0.47	9.52 ± 0.37	10.46 ± 0.39	10.27 ± 0.41	10.38 ± 0.39	9.93 ± 0.37	10.56 ± 0.37
Week 13	9.06 ± 0.42	9.91 ± 0.57	9.62 ± 0.77	9.27 ± 0.35	9.01 ± 0.46	9.54 ± 0.57	10.42 ± 0.53
Segmented neutrophils ($10^3/\mu L$)							
Day 3	1.65 ± 0.15	1.45 ± 0.18	1.39 ± 0.11	1.38 ± 0.13	1.42 ± 0.14	1.76 ± 0.25	1.41 ± 0.14
Day 23	1.20 ± 0.13	1.24 ± 0.13	1.08 ± 0.09	1.15 ± 0.11	1.18 ± 0.11	1.25 ± 0.13	1.23 ± 0.12
Week 13	1.87 ± 0.16	2.20 ± 0.21	1.92 ± 0.25	1.61 ± 0.19	1.76 ± 0.17	1.49 ± 0.22▲	1.92 ± 0.10
Lymphocytes ($10^3/\mu L$)							
Day 3	7.08 ± 0.43	7.78 ± 0.39	7.54 ± 0.24	7.43 ± 0.39	8.61 ± 0.34*	8.19 ± 0.21*	9.10 ± 0.42**
Day 23	8.46 ± 0.46	8.23 ± 0.42	9.29 ± 0.40	9.03 ± 0.39	9.13 ± 0.31	8.62 ± 0.32	9.28 ± 0.38
Week 13	7.13 ± 0.37	7.63 ± 0.39	7.63 ± 0.55	7.60 ± 0.31	7.21 ± 0.34	7.99 ± 0.46	8.38 ± 0.47
Monocytes ($10^3/\mu L$)							
Day 3	0.06 ± 0.02	0.02 ± 0.01	0.07 ± 0.03	0.07 ± 0.02	0.05 ± 0.03	0.10 ± 0.02▲	0.08 ± 0.03
Day 23	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.06 ± 0.03	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
Week 13	0.03 ± 0.01	0.06 ± 0.03	0.04 ± 0.03	0.05 ± 0.03	0.01 ± 0.01	0.01 ± 0.01	0.06 ± 0.03
Eosinophils ($10^3/\mu L$)							
Day 3	0.01 ± 0.01	0.03 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.04 ± 0.02
Day 23	0.06 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.01 ± 0.01
Week 13	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	0.04 ± 0.02	0.05 ± 0.02	0.06 ± 0.02

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Female (continued)							
Clinical Chemistry							
n	10	10	10	10	10	10	10
Urea nitrogen (mg/dL)							
Day 3	22.0 ± 1.0	20.0 ± 0.8	21.1 ± 0.8	18.9 ± 0.9	21.8 ± 0.8	20.3 ± 0.9	19.8 ± 1.5
Day 23	22.5 ± 1.1	22.6 ± 0.8	19.6 ± 0.7	19.8 ± 0.8	20.1 ± 0.3	19.2 ± 0.7▲	21.2 ± 1.1
Week 13	25.0 ± 0.8	24.7 ± 0.8	23.0 ± 1.1	25.3 ± 0.7	26.5 ± 1.2	23.7 ± 0.7	23.9 ± 0.6
Creatinine (mg/dL)							
Day 3	0.47 ± 0.02	0.45 ± 0.02	0.46 ± 0.02	0.42 ± 0.02	0.47 ± 0.02	0.41 ± 0.02	0.46 ± 0.03
Day 23	0.54 ± 0.03	0.52 ± 0.03	0.48 ± 0.03	0.47 ± 0.02	0.49 ± 0.01	0.48 ± 0.03	0.50 ± 0.03
Week 13	0.59 ± 0.02	0.65 ± 0.02	0.60 ± 0.02	0.63 ± 0.02	0.65 ± 0.04	0.63 ± 0.02	0.59 ± 0.02
Total protein (g/dL)							
Day 3	5.9 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.7 ± 0.1	6.0 ± 0.0	5.7 ± 0.0	5.9 ± 0.1
Day 23	6.2 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.2 ± 0.1
Week 13	6.7 ± 0.1	6.7 ± 0.1	6.4 ± 0.3	6.4 ± 0.1	6.7 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
Albumin (g/dL)							
Day 3	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.0	3.2 ± 0.1	3.2 ± 0.0	3.2 ± 0.1
Day 23	3.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.4 ± 0.1	3.5 ± 0.1
Week 13	4.2 ± 0.1	4.0 ± 0.1	3.9 ± 0.2*	3.8 ± 0.1**	4.0 ± 0.1**	3.8 ± 0.1**	3.8 ± 0.1**
Alanine aminotransferase (IU/L)							
Day 3	37 ± 2	36 ± 1	39 ± 1	39 ± 2	38 ± 1	40 ± 1	40 ± 1
Day 23	36 ± 1	37 ± 2	34 ± 1	35 ± 1	35 ± 1	36 ± 0	37 ± 1
Week 13	40 ± 1	38 ± 1	37 ± 2	41 ± 2	44 ± 3	41 ± 1	42 ± 2
Alkaline phosphatase (IU/L)							
Day 3	576 ± 13	575 ± 12	592 ± 12	584 ± 14	597 ± 13	574 ± 16	560 ± 13
Day 23	383 ± 12	380 ± 11	367 ± 7	396 ± 9	359 ± 7*	355 ± 4**▲	347 ± 9**▲
Week 13	239 ± 7	220 ± 7	226 ± 7[b]	231 ± 6	197 ± 11	262 ± 9▲	188 ± 9*
Creatine kinase (IU/L)							
Day 3	358 ± 41	307 ± 37[b]	361 ± 48[b]	319 ± 33	344 ± 32	325 ± 73	387 ± 47[b]
Day 23	281 ± 77	228 ± 35	299 ± 41	213 ± 43	242 ± 44	325 ± 86	457 ± 71▲[b]
Week 13	215 ± 53	213 ± 21	185 ± 42	237 ± 53	264 ± 72	179 ± 33	290 ± 51
Sorbitol dehydrogenase (IU/L)							
Day 3	7 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 0	6 ± 0
Day 23	8 ± 1	7 ± 1	7 ± 0	8 ± 1	8 ± 0	7 ± 0	6 ± 0*
Week 13	6 ± 0	6 ± 1	7 ± 1	5 ± 0	5 ± 0	6 ± 0	5 ± 0
Bile acids (μmol/L)							
Day 3	27.5 ± 3.6	28.5 ± 3.9	37.4 ± 3.1	33.4 ± 3.3	29.4 ± 2.8	35.6 ± 4.0	38.2 ± 1.8*
Day 23	40.9 ± 6.1	47.7 ± 6.4	48.7 ± 5.7	44.7 ± 7.9	43.7 ± 6.0	52.2 ± 7.1	61.6 ± 6.0
Week 13	35.1 ± 3.9	40.7 ± 4.3	35.6 ± 4.7	56.0 ± 9.2	31.7 ± 2.7	29.9 ± 3.9	25.1 ± 3.8▲▲

* Significantly different (P≤0.05) from the untreated control group by Dunn's or Shirley's test
** Significantly different (P≤0.01) from the untreated control group by Shirley's test
▲ Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
▲▲ P≤0.01
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=8

TABLE B2
Urinalysis and Urinary Metabolite Data for Male Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Vehicle Control	5,000 ppm	20,000 ppm	80,000 ppm
n	5	5	5	5
Urine volume (mL/24 hours)				
Day 28	13.0 ± 1.5	14.2 ± 0.8	11.2 ± 1.1	11.8 ± 0.9
Day 84	9.9 ± 0.3	10.5 ± 0.6	8.0 ± 0.7	7.6 ± 1.4
Urine creatinine (mg/dL)				
Day 28	95.0 ± 8.4	84.0 ± 5.3	111.0 ± 7.8	105.0 ± 7.1
Day 84	147.0 ± 2.5	138.0 ± 6.0	165.0 ± 13.7	163.0 ± 25.9
Trichloroacetic acid (μg/mg creatinine)				
Day 28	2.39 ± 0.19	8.05 ± 0.43**	38.52 ± 4.09**	86.95 ± 9.57**
Day 84	0.778 ± 0.025[b]	12.874 ± 1.533*	15.772 ± 1.933**	73.337 ± 15.265**
Free trichloroethanol (μg/mg creatinine)				
Day 28	0.67 ± 0.37[b]	32.50 ± 17.24*	115.59 ± 42.78**	139.79 ± 97.14**
Total trichloroethanol (μg/mg creatinine)				
Day 28	3.3 ± 0.2	156.0 ± 15.3**	629.5 ± 115.5**	1,085.7 ± 81.3**
Day 84	2.6 ± 0.1	136.1 ± 14.9**	315.9 ± 24.5**	1,147.8 ± 105.4**

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=4

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

TABLE C1 **Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane** **C-2**
TABLE C2 **Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane** **C-3**

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
n	10	10	10	10	10	10	10
Male							
Necropsy body wt	310 ± 4	326 ± 7*	321 ± 3	332 ± 4**	325 ± 4*	310 ± 4▲	296 ± 6▲▲
Heart							
Absolute	1.049 ± 0.018	1.125 ± 0.023*	1.085 ± 0.038	1.063 ± 0.027	1.073 ± 0.027	1.037 ± 0.024▲	1.002 ± 0.022▲▲
Relative	3.39 ± 0.05	3.47 ± 0.12	3.38 ± 0.11	3.20 ± 0.07	3.30 ± 0.08	3.34 ± 0.06	3.39 ± 0.07
R. Kidney							
Absolute	1.141 ± 0.028	1.152 ± 0.029	1.143 ± 0.018	1.217 ± 0.021	1.164 ± 0.039	1.153 ± 0.030	1.116 ± 0.037
Relative	3.69 ± 0.08	3.53 ± 0.06	3.57 ± 0.06	3.67 ± 0.04	3.58 ± 0.10	3.71 ± 0.07	3.77 ± 0.09
Liver							
Absolute	12.059 ± 0.272	13.050 ± 0.404	13.181 ± 0.262	14.206 ± 0.262**	13.294 ± 0.446	12.469 ± 0.419	11.370 ± 0.391▲▲
Relative	38.94 ± 0.61	39.94 ± 0.59	41.11 ± 0.67	42.81 ± 0.76*	40.87 ± 1.11	40.14 ± 1.02	38.41 ± 0.89
Lung							
Absolute	1.629 ± 0.046	1.701 ± 0.081	1.697 ± 0.067	1.807 ± 0.069	1.765 ± 0.100	1.690 ± 0.059	1.660 ± 0.097
Relative	5.27 ± 0.16	5.20 ± 0.20	5.29 ± 0.20	5.45 ± 0.22	5.44 ± 0.32	5.46 ± 0.23	5.60 ± 0.27
R. Testis							
Absolute	1.497 ± 0.032	1.455 ± 0.026	1.478 ± 0.013	1.474 ± 0.024	1.425 ± 0.018	1.500 ± 0.026	1.438 ± 0.036
Relative	4.84 ± 0.10	4.47 ± 0.07**	4.61 ± 0.06	4.44 ± 0.05**	4.39 ± 0.07**	4.84 ± 0.09▲▲	4.86 ± 0.08▲▲
Thymus							
Absolute	0.310 ± 0.020	0.330 ± 0.016	0.290 ± 0.016	0.304 ± 0.017	0.306 ± 0.012	0.291 ± 0.011	0.299 ± 0.019
Relative	1.00 ± 0.05	1.01 ± 0.04	0.91 ± 0.05	0.92 ± 0.05	0.94 ± 0.04	0.94 ± 0.03	1.01 ± 0.06
Female							
Necropsy body wt	184 ± 2	184 ± 3	188 ± 3	184 ± 3	173 ± 3	181 ± 2	175 ± 2*▲
Heart							
Absolute	0.696 ± 0.017	0.723 ± 0.014	0.702 ± 0.032	0.701 ± 0.019	0.634 ± 0.025	0.675 ± 0.027	0.684 ± 0.042
Relative	3.79 ± 0.10	3.94 ± 0.09	3.73 ± 0.15	3.81 ± 0.09	3.66 ± 0.11	3.72 ± 0.13	3.91 ± 0.22
R. Kidney							
Absolute	0.709 ± 0.014	0.716 ± 0.015	0.698 ± 0.015	0.711 ± 0.012	0.692 ± 0.023	0.685 ± 0.009	0.649 ± 0.015**▲▲
Relative	3.86 ± 0.07	3.90 ± 0.06	3.71 ± 0.06	3.86 ± 0.05	4.00 ± 0.11	3.79 ± 0.06	3.72 ± 0.06
Liver							
Absolute	6.090 ± 0.179	6.187 ± 0.156	6.513 ± 0.308	6.333 ± 0.125	5.744 ± 0.180	6.067 ± 0.188	5.151 ± 0.086**▲▲
Relative	33.14 ± 0.96	33.69 ± 0.65	34.67 ± 1.71	34.41 ± 0.43	33.19 ± 0.65	33.46 ± 0.89	29.53 ± 0.38*▲▲
Lung							
Absolute	1.135 ± 0.051	1.119 ± 0.020	1.119 ± 0.034	1.134 ± 0.039	1.047 ± 0.026	1.133 ± 0.047	1.109 ± 0.030
Relative	6.17 ± 0.28	6.11 ± 0.17	5.96 ± 0.18	6.16 ± 0.19	6.07 ± 0.15	6.25 ± 0.24	6.37 ± 0.20
Thymus							
Absolute	0.248 ± 0.014	0.237 ± 0.010	0.235 ± 0.009	0.251 ± 0.014	0.234 ± 0.010	0.263 ± 0.010	0.246 ± 0.013
Relative	1.35 ± 0.07	1.29 ± 0.06	1.25 ± 0.05	1.37 ± 0.08	1.35 ± 0.05	1.45 ± 0.05	1.41 ± 0.07

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

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Untreated Control	Vehicle Control	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Male							
n	9	10	10	10	10	10	10
Necropsy body wt	31.7 ± 0.7	32.7 ± 1.3	30.5 ± 0.7	30.6 ± 0.9	30.4 ± 0.7	30.0 ± 0.5▲	28.0 ± 0.4**▲▲
Heart							
Absolute	0.161 ± 0.007	0.194 ± 0.007**	0.167 ± 0.006▲▲	0.174 ± 0.008▲▲	0.168 ± 0.003▲▲	0.159 ± 0.005▲▲	0.150 ± 0.004▲▲
Relative	5.08 ± 0.18	5.97 ± 0.19**	5.46 ± 0.15	5.69 ± 0.24	5.54 ± 0.10	5.30 ± 0.12▲▲	5.36 ± 0.16▲▲
R. Kidney							
Absolute	0.289 ± 0.013	0.333 ± 0.008**	0.310 ± 0.009▲	0.312 ± 0.006▲	0.310 ± 0.006▲	0.312 ± 0.008▲	0.296 ± 0.006▲▲
Relative	9.11 ± 0.33	10.32 ± 0.43*	10.17 ± 0.20*	10.29 ± 0.40*	10.23 ± 0.34*	10.40 ± 0.26**	10.58 ± 0.20**
Liver							
Absolute	1.676 ± 0.045	1.855 ± 0.046*	1.717 ± 0.039	1.752 ± 0.044	1.747 ± 0.040	1.828 ± 0.061	1.646 ± 0.040▲▲
Relative	52.90 ± 1.00	57.39 ± 2.15	56.35 ± 0.68	57.79 ± 2.49*	57.46 ± 0.86*	60.85 ± 1.54**	58.71 ± 1.36**
Lung							
Absolute	0.213 ± 0.012	0.251 ± 0.008*	0.237 ± 0.011[b]	0.236 ± 0.016	0.234 ± 0.013	0.237 ± 0.015	0.224 ± 0.014
Relative	6.69 ± 0.26	7.80 ± 0.40*	7.82 ± 0.32[b]	7.74 ± 0.50	7.71 ± 0.47	7.88 ± 0.49	8.02 ± 0.53
R. Testis							
Absolute	0.128 ± 0.004	0.129 ± 0.004	0.125 ± 0.004	0.125 ± 0.003	0.130 ± 0.003	0.131 ± 0.005	0.126 ± 0.002
Relative	4.06 ± 0.08	4.01 ± 0.27	4.11 ± 0.10	4.13 ± 0.17	4.30 ± 0.14	4.36 ± 0.14	4.49 ± 0.10*
Thymus							
Absolute	0.060 ± 0.005	0.053 ± 0.003	0.049 ± 0.006	0.044 ± 0.002	0.045 ± 0.003	0.051 ± 0.005	0.049 ± 0.005
Relative	1.89 ± 0.16	1.65 ± 0.11	1.61 ± 0.18	1.46 ± 0.08	1.49 ± 0.10	1.71 ± 0.17	1.73 ± 0.19
Female							
n	10	10	10	10	10	10	8
Necropsy body wt	26.8 ± 0.9	28.1 ± 0.8	26.8 ± 0.6	26.8 ± 0.8	25.3 ± 0.8▲▲	25.0 ± 0.7▲▲	24.1 ± 0.5*▲▲
Heart							
Absolute	0.139 ± 0.005	0.138 ± 0.003	0.156 ± 0.010	0.150 ± 0.012	0.129 ± 0.006	0.129 ± 0.005	0.125 ± 0.007
Relative	5.22 ± 0.23	4.94 ± 0.14	5.82 ± 0.36	5.61 ± 0.41	5.12 ± 0.17	5.19 ± 0.20	5.18 ± 0.18
R. Kidney							
Absolute	0.192 ± 0.005	0.215 ± 0.005**	0.205 ± 0.003	0.205 ± 0.004	0.201 ± 0.004▲	0.193 ± 0.004▲▲	0.195 ± 0.002▲▲
Relative	7.19 ± 0.17	7.67 ± 0.17	7.70 ± 0.24	7.67 ± 0.18	8.00 ± 0.24*	7.75 ± 0.24*	8.11 ± 0.16**
Liver							
Absolute	1.343 ± 0.036	1.415 ± 0.042	1.385 ± 0.039	1.381 ± 0.038	1.276 ± 0.039▲	1.258 ± 0.045▲	1.243 ± 0.051▲▲
Relative	50.17 ± 0.94	50.54 ± 1.33	51.84 ± 1.43	51.58 ± 1.04	50.56 ± 0.72	50.38 ± 1.46	51.52 ± 1.71
Lung							
Absolute	0.255 ± 0.021	0.262 ± 0.010	0.277 ± 0.018	0.263 ± 0.024	0.231 ± 0.012	0.221 ± 0.011	0.240 ± 0.022
Relative	9.53 ± 0.79	9.37 ± 0.38	10.37 ± 0.65	9.87 ± 0.95	9.19 ± 0.49	8.88 ± 0.47	9.93 ± 0.79
Thymus							
Absolute	0.057 ± 0.003	0.058 ± 0.004	0.055 ± 0.003[b]	0.052 ± 0.002	0.063 ± 0.005	0.046 ± 0.001▲	0.053 ± 0.003
Relative	2.12 ± 0.13	2.07 ± 0.14	2.06 ± 0.09[b]	1.93 ± 0.06	2.48 ± 0.15▲	1.85 ± 0.08	2.18 ± 0.08

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

APPENDIX D
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE D1 Summary of Reproductive Tissue Evaluations in Male Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane D-2
TABLE D2 Summary of Estrous Cycle Characterization in Female Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane D-2
TABLE D3 Summary of Reproductive Tissue Evaluations in Male Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane D-3
TABLE D4 Summary of Estrous Cycle Characterization in Female Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane D-3

TABLE D1
Summary of Reproductive Tissue Evaluations in Male Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Vehicle Control	20,000 ppm	40,000 ppm	80,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	326 ± 7	325 ± 4	310 ± 4*	296 ± 6**
L. epididymis	0.460 ± 0.007	0.456 ± 0.006	0.446 ± 0.005	0.442 ± 0.009
L. cauda epididymis	0.195 ± 0.003	0.188 ± 0.004	0.189 ± 0.006	0.188 ± 0.004
L. testis	1.51 ± 0.01	1.52 ± 0.02	1.53 ± 0.03	1.46 ± 0.04
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.33 ± 0.18	8.89 ± 0.34	9.13 ± 0.44	9.68 ± 0.32
Spermatid heads (10^7/testis)	14.06 ± 0.22	13.46 ± 0.50	13.97 ± 0.64	14.20 ± 0.65
Spermatid count (mean/10^{-4} mL suspension)	70.30 ± 1.08	67.28 ± 2.48	69.83 ± 3.20	70.98 ± 3.26
Epididymal spermatozoal measurements				
Motility (%)	98.94 ± 0.10	98.79 ± 0.15	98.75 ± 0.23	98.38 ± 0.48
Concentration (10^6/g cauda epididymal tissue)	755 ± 19	713 ± 18	722 ± 22	677 ± 15*

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

TABLE D2
Summary of Estrous Cycle Characterization in Female Rats in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Vehicle Control	20,000 ppm	40,000 ppm	80,000 ppm
n	10	10	10	10
Necropsy body weight (g)	184 ± 3	173 ± 3	181 ± 2	175 ± 2*
Estrous cycle length (days)	5.80 ± 0.32	5.25 ± 0.26	5.94 ± 0.34[b]	5.85 ± 0.29
Estrous stages (% of cycle)				
Diestrus	33.3	33.3	45.0	40.0
Proestrus	17.5	15.0	13.3	18.3
Estrus	35.0	33.3	25.8	23.3
Metestrus	14.2	18.3	15.8	16.7
Uncertain diagnosis	0.0	0.0	0.0	1.7

* Significantly different (P≤0.05) from the vehicle control group by Williams' test
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the vehicle control group in the relative length of time spent in the estrous stages.
[b] Estrous cycle length was longer than 12 days or unclear in 1 of 10 animals.

TABLE D3
Summary of Reproductive Tissue Evaluations in Male Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Vehicle Control	20,000 ppm	40,000 ppm	80,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	32.7 ± 1.3	30.4 ± 0.7	30.0 ± 0.5*	28.0 ± 0.4**
L. epididymis	0.047 ± 0.001	0.045 ± 0.002	0.045 ± 0.001	0.049 ± 0.002
L. cauda epididymis	0.018 ± 0.001	0.017 ± 0.001	0.018 ± 0.001	0.019 ± 0.001
L. testis	0.121 ± 0.004	0.120 ± 0.003	0.118 ± 0.002	0.119 ± 0.001
Spermatid measurements				
Spermatid heads (10^7/g testis)	16.88 ± 1.23	15.46 ± 0.86	17.05 ± 0.95	17.00 ± 0.77
Spermatid heads (10^7/testis)	2.03 ± 0.16	1.85 ± 0.10	2.00 ± 0.11	2.02 ± 0.09
Spermatid count (mean/10^{-4} mL suspension)	63.48 ± 4.87	57.70 ± 3.22	62.58 ± 3.28	63.05 ± 2.69
Epididymal spermatozoal measurements				
Motility (%)	98.75 ± 0.11	98.34 ± 0.48	98.26 ± 0.21	98.81 ± 0.10
Concentration (10^6/g cauda epididymal tissue)	1,632 ± 100	1,408 ± 73	1,429 ± 68	1,292 ± 56*

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

TABLE D4
Summary of Estrous Cycle Characterization in Female Mice in the 13-Week Feed Study of 1,1,1-Trichloroethane[a]

	Vehicle Control	20,000 ppm	40,000 ppm	80,000 ppm
n	10	10	10	8
Necropsy body weight (g)	28.1 ± 0.8	25.3 ± 0.8**	25.0 ± 0.7**	24.1 ± 0.5**
Estrous cycle length (days)	4.25 ± 0.19	4.72 ± 0.55[b]	4.20 ± 0.11	4.06 ± 0.06
Estrous stages (% of cycle)				
Diestrus	33.3	38.3	26.7	28.1
Proestrus	20.8	18.3	22.5	19.8
Estrus	30.0	25.0	30.8	30.2
Metestrus	15.8	18.3	20.0	21.9

** Significantly different (P≤0.01) from the vehicle control group by Williams' test
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the vehicle control group in the relative length of time spent in the estrous stages.
[b] Estrous cycle length was longer than 12 days or unclear in 1 of 10 animals.

APPENDIX E
GENETIC TOXICOLOGY

TABLE E1 Mutagenicity of 1,1,1-Trichloroethane in *Salmonella typhimurium* E-2
TABLE E2 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane E-6
TABLE E3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 1,1,1-Trichloroethane E-18
TABLE E4 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 1,1,1-Trichloroethane E-19
TABLE E5 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of 1,1,1-Trichloroethane in Feed for 13 Weeks E-20

TABLE E1
Mutagenicity of 1,1,1-Trichloroethane in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/plate[a]		
		−S9	+S9	
			10% hamster	10% rat
Study Performed at SRI International[b]				
TA100	0	108 ± 7.4	144 ± 9.6	108 ± 7.4
	33	157 ± 8.9	149 ± 6.3	144 ± 7.1
	100	152 ± 5.7	148 ± 3.0	133 ± 8.5
	333	126 ± 12.1	127 ± 13.5	133 ± 3.5
	1,000	123 ± 8.2	110 ± 7.8	87 ± 4.3
	3,333	27 ± 17.0[c]	61 ± 28.3[c]	81 ± 12.2
Trial summary		Negative	Negative	Negative
Positive control[d]		261 ± 15.6	681 ± 11.1	261 ± 15.6
TA1535	0	10 ± 2.7	21 ± 9.1	28 ± 2.3
	33	17 ± 3.8	9 ± 1.7	8 ± 4.0
	100	12 ± 2.7	7 ± 2.9	9 ± 1.9
	333	9 ± 2.6	6 ± 0.3	7 ± 0.3
	1,000	2 ± 0.7	4 ± 1.5	3 ± 0.6[c]
	3,333	Toxic	2 ± 1.2	4 ± 0.9[c]
Trial summary		Negative	Negative	Negative
Positive control		278 ± 3.5	356 ± 7.7	161 ± 4.6
TA1537	0	6 ± 0.7	12 ± 2.3	7 ± 2.9
	33	7 ± 1.2	7 ± 1.2	9 ± 2.0
	100	8 ± 2.7	6 ± 1.8	9 ± 1.7
	333	5 ± 1.8	7 ± 0.3	8 ± 1.9
	1,000	4 ± 0.6	7 ± 3.2	4 ± 1.9
	3,333	Toxic	1 ± 0.7[c]	4 ± 1.3
Trial summary		Negative	Negative	Negative
Positive control		117 ± 11.9	344 ± 12.5	134 ± 14.2
TA98	0	14 ± 2.4	35 ± 4.5	65 ± 7.3
	33	22 ± 3.3	23 ± 3.0	29 ± 6.7
	100	14 ± 3.5	21 ± 2.6	25 ± 2.2
	333	14 ± 1.9	25 ± 3.8	26 ± 3.8
	1,000	12 ± 1.5	20 ± 4.0	19 ± 3.5
	3,333	Toxic	17 ± 0.6	20 ± 3.5
Trial summary		Negative	Negative	Negative
Positive control		821 ± 18.2	551 ± 44.2	239 ± 17.1

TABLE E1
Mutagenicity of 1,1,1-Trichloroethane in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/plate		
		−S9	+S9	
			10% hamster	10% rat
Study Performed at Case Western Reserve University[b]				
TA100	0	199 ± 4.4	271 ± 5.9	240 ± 16.3
	100	176 ± 13.4	323 ± 9.1	284 ± 6.6
	333	193 ± 6.1	335 ± 23.0	292 ± 15.6
	1,000	204 ± 15.2	300 ± 12.9	290 ± 11.1
	3,333	203 ± 33.3	292 ± 5.9	287 ± 15.9
	10,000	180 ± 13.4	165 ± 30.4	293 ± 18.9
Trial summary		Negative	Negative	Negative
Positive control		867 ± 63.7	1,905 ± 56.4	1,126 ± 47.1
TA1535	0	6 ± 0.3	7 ± 2.0	6 ± 1.3
	100	4 ± 0.7	7 ± 1.9	7 ± 1.3
	333	5 ± 0.3	7 ± 1.2	7 ± 0.0
	1,000	4 ± 0.7	7 ± 1.5	8 ± 1.3
	3,333	3 ± 0.0	5 ± 1.5	5 ± 1.2
	10,000	1 ± 0.0	4 ± 2.5	3 ± 0.6
Trial summary		Negative	Negative	Negative
Positive control		936 ± 79.2	116 ± 16.2	99 ± 12.2
TA1537	0	5 ± 1.2	8 ± 0.9	13 ± 1.5
	100	4 ± 0.7	7 ± 1.9	11 ± 2.7
	333	6 ± 0.7	9 ± 1.5	12 ± 2.7
	1,000	4 ± 0.9	9 ± 0.6	11 ± 4.2
	3,333	5 ± 0.6	4 ± 0.9	3 ± 0.3
	10,000	4 ± 0.0	4 ± 0.9	4 ± 2.0
Trial summary		Negative	Negative	Negative
Positive control		83 ± 9.3	163 ± 9.5	120 ± 1.2
TA98	0	22 ± 1.5	23 ± 3.2	29 ± 1.2
	33		27 ± 2.2	
	100	21 ± 1.8	26 ± 4.7	28 ± 0.7
	333	24 ± 3.6	29 ± 0.9	31 ± 1.9
	1,000	20 ± 1.0	28 ± 3.3	35 ± 2.4
	3,333	24 ± 1.3	30 ± 1.8	26 ± 4.9
	10,000	22 ± 2.4		6 ± 3.3
Trial summary		Negative	Negative	Negative
Positive control		464 ± 49.0	1,946 ± 183.3	645 ± 12.6

TABLE E1
Mutagenicity of 1,1,1-Trichloroethane in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/plate		
		−S9	+S9	
			10% hamster	10% rat
Study Performed at SRI International[e]				
TA100	0	143 ± 7.1	116 ± 3.8	132 ± 7.8
	10		136 ± 7.0	
	33	132 ± 4.1	134 ± 9.7	118 ± 2.9
	100	153 ± 11.7	109 ± 5.1	127 ± 5.7
	333	134 ± 5.4	124 ± 5.6	121 ± 6.2
	1,000	132 ± 6.8	118 ± 12.3	113 ± 7.6
	3,333	0 ± 0.0[c]		109 ± 4.1
Trial summary		Negative	Negative	Negative
Positive control		377 ± 8.7	966 ± 42.3	456 ± 24.8
TA1535	0	18 ± 0.9	17 ± 0.0	27 ± 5.8
	10		22 ± 6.8	
	33	18 ± 0.9	20 ± 3.1	34 ± 5.8
	100	24 ± 2.3	24 ± 4.8	35 ± 2.7
	333	14 ± 3.8	21 ± 3.0	29 ± 4.0
	1,000	5 ± 5.3[c]	19 ± 4.1	19 ± 0.9
	3,333	0 ± 0.0[c]		11 ± 5.8[c]
Trial summary		Negative	Negative	Negative
Positive control		342 ± 42.1	245 ± 10.7	178 ± 24.1
TA1537	0	5 ± 1.9	5 ± 0.9	6 ± 0.9
	10		4 ± 0.9	
	33	8 ± 4.0	6 ± 0.9	5 ± 0.6
	100	7 ± 1.9	4 ± 1.2	6 ± 1.8
	333	4 ± 0.9	3 ± 1.3	6 ± 1.2
	1,000	6 ± 3.8	5 ± 1.0	10 ± 2.0
	3,333	0 ± 0.0[c]		4 ± 1.2[c]
Trial summary		Negative	Negative	Negative
Positive control		350 ± 41.7	406 ± 11.5	135 ± 8.7
TA98	0	27 ± 1.0	20 ± 3.6	28 ± 2.3
	10		20 ± 2.5	
	33	20 ± 4.4	25 ± 3.8	32 ± 0.6
	100	17 ± 0.7	28 ± 2.3	34 ± 8.4
	333	24 ± 0.3	28 ± 2.2	36 ± 0.3
	1,000	7 ± 4.3	27 ± 4.4	32 ± 2.5
	3,333	0 ± 0.0[c]		30 ± 1.5
Trial summary		Negative	Negative	Negative
Positive control		575 ± 33.3	764 ± 21.3	326 ± 10.7

TABLE E1
Mutagenicity of 1,1,1-Trichloroethane in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/plate		
		−S9	+S9	
			10% hamster	10% rat
Study Performed at Case Western Reserve University[e]				
TA100	0	98 ± 10.9	162 ± 18.2	118 ± 2.0
	100	77 ± 8.4	98 ± 4.8	112 ± 2.6
	333	92 ± 4.8	115 ± 22.2	92 ± 12.0
	1,000	81 ± 4.3	122 ± 3.8	114 ± 11.9
	3,333	80 ± 6.1	112 ± 3.5	115 ± 17.6
	10,000	68 ± 3.9	104 ± 1.5	110 ± 9.9
Trial summary		Negative	Negative	Negative
Positive control		1,171 ± 97.5	1,320 ± 173.4	1,671 ± 555.4
TA1535	0	3 ± 1.3	5 ± 1.3	5 ± 1.5
	100	4 ± 0.3	6 ± 1.2	3 ± 1.3
	333	3 ± 0.0	7 ± 1.9	3 ± 0.3
	1,000	2 ± 1.2	5 ± 0.9	3 ± 0.6
	3,333	4 ± 0.9	4 ± 0.0	4 ± 1.2
	10,000	2 ± 0.7	5 ± 1.5	4 ± 0.9
Trial summary		Negative	Negative	Negative
Positive control		354 ± 52.9	289 ± 50.3	88 ± 15.2
TA1537	0	4 ± 1.2	3 ± 0.6	4 ± 1.5
	100	3 ± 0.9	3 ± 1.2	1 ± 0.7
	333	3 ± 1.0	4 ± 0.9	3 ± 0.3
	1,000	2 ± 0.3	5 ± 1.3	2 ± 0.7
	3,333	3 ± 1.5	2 ± 0.6	2 ± 0.9
	10,000	1 ± 0.9	1 ± 0.3	5 ± 1.2
Trial summary		Negative	Negative	Negative
Positive control		129 ± 38.7	137 ± 14.4	297 ± 42.7
TA98	0	12 ± 1.5	15 ± 1.5	17 ± 1.2
	100	7 ± 4.5	18 ± 2.4	21 ± 0.3
	333	9 ± 0.9	19 ± 2.6	16 ± 3.3
	1,000	11 ± 1.5	15 ± 0.9	13 ± 1.9
	3,333	9 ± 0.9	15 ± 1.5	14 ± 2.4
	10,000	10 ± 1.5	10 ± 2.0	14 ± 2.6
Trial summary		Negative	Negative	Negative
Positive control		165 ± 30.1	615 ± 107.5	838 ± 59.6

[a] Revertants are presented as mean ± standard error from three plates. 0 μg/plate is the solvent control.
[b] The detailed protocol and these data are presented by Haworth *et al*. (1983).
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[e] The detailed protocol and these data are presented by Zeiger *et al*. (1987).

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane[a]

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction
Study Performed at SRI International[c]						
-S9						
Trial 1						
Dimethylsulfoxide[d]						
		73	98	141	64	
		70	82	185	88	
		74	100	179	80	
		76	120	143	63	74
Ethyl methanesulfonate[e]						
	500 μg/mL	47	44	865	618	
		29	24	773	883	
		29	26	657	751	751*
1,1,1-Trichloroethane						
	0.26	62	66	90	48	
		56	54	91	54	51
	0.33	94	72	81	29	
		92	75	83	30	30
	0.41	89	95	54	20	
	0.51	91	16	71	26	
		65	18	77	40	33
	0.64	Lethal				
		Lethal				

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Study Performed at SRI International (continued)						
-S9 (continued)						
Trial 2						
Dimethylsulfoxide						
		83	85	76	30	
		87	115	67	26	28
Ethyl methanesulfonate						
	500 μg/mL	60	49	534	296	
		49	30	975	663	
		50	40	995	668	542*
1,1,1-Trichloroethane						
	0.21	67	56	102	51	
		48	42	44	30	41
	0.26	47	48	35	25	
		82	70	82	33	29
	0.33	46	41	57	41	
		83	75	71	29	35
	0.41	68	53	77	38	
		67	53	36	18	28
	0.64	91	45	80	29	
		Lethal				
	0.80	Lethal				
		Lethal				

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Study Performed at SRI International (continued)						
-S9 (continued)						
Trial 3						
Dimethylsulfoxide						
		56	92	60	36	
		64	108	33	17	
		62	98	64	34	
		62	102	49	26	28
Ethyl methanesulfonate						
	500 μg/mL	43	48	649	501	
		37	47	659	588	
		41	46	720	585	558*
1,1,1-Trichloroethane						
	0.21	56	56	42	25	
		59	58	43	24	
		74	111	52	23	24
	0.26	62	76	40	22	
		63	82	52	28	
		67	89	35	17	22
	0.33	61	60	72	39	
		68	79	41	20	
		62	72	39	21	27
	0.41	57	52	49	29	
		59	47	75	42	
		62	44	62	33	35
	0.51	68	41	59	29	
		39	19	22	19	
		76	49	47	21	23
	0.64	Lethal				
		Lethal				
		Lethal				

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Study Performed at SRI International (continued)						
+S9						
Trial 1						
Dimethylsulfoxide						
		73	94	146	67	
		91	110	154	57	
		79	96	109	46	57
Methylcholanthrene[e]						
	5 μg/mL	34	20	327	325	
		36	21	362	340	
		35	19	395	382	349*
1,1,1-Trichloroethane						
	0.21	91	89	105	38	
		61	72	68	37	38
	0.26	94	83	54	19	
		77	68	127	55	37
	0.41	71	54	74	35	
		62	54	86	46	41
	0.51	80	49	159	67	
		68	56	153	75	71
	0.64	65	15	236	120	
		77	23	210	91	106*

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Study Performed at SRI International (continued)						
+S9 (continued)						
Trial 2						
Dimethylsulfoxide						
		93	108	135	48	
		72	93	73	34	
		88	103	116	44	
		75	96	83	37	41
Methylcholanthrene						
	5 μg/mL	50	39	413	276	
		43	33	356	279	
		43	35	405	316	291*
1,1,1-Trichloroethane						
	0.21	60	59	75	42	
		65	66	55	28	
		77	79	99	43	37
	0.26	70	67	93	44	
		66	57	52	26	
		66	61	81	41	37
	0.33	61	51	42	23	
		66	52	83	42	
		74	63	89	40	35
	0.41	77	53	83	36	
		68	50	43	21	
		56	35	79	47	35
	0.51	58	21	62	35	
		62	31	101	55	
		67	27	92	46	45
	0.64	Lethal				
		Lethal				
		Lethal				

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Study Performed at Litton Bionetics, Inc.[f]						
-S9						
Trial 1						
Dimethylsulfoxide						
		117	123	87	25	
		80	77	58	24	
		97	100	41	14	21
Ethyl methanesulfonate						
	500 μg/mL	23	6	727	1,054	
		11	4	413	1,311	
		27	13	719	887	1,084*
1,1,1-Trichloroethane						
	0.05	110	110	47	14	
		94	78	66	23	
		67	65	30	15	18
	0.1	91	102	41	15	
		106	100	34	11	
		93	78	42	15	13
	0.2	114	98	51	15	
		115	94	45	13	14
	0.4	Lethal				
		Lethal				
		Lethal				

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Study Performed at Litton Bionetics, Inc. (continued)						
-S9 (continued)						
Trial 2						
Dimethylsulfoxide						
		95	92	63	22	
		111	111	69	21	
		102	87	78	26	
		112	110	114	34	26
Ethyl methanesulfonate						
	250 μg/mL	94	51	961	341	
		92	58	1,115	404	
		81	48	917	380	375*
1,1,1-Trichloroethane						
	0.05	113	109	42	12	
		117	82	85	24	
		108	99	88	27	21
	0.1	103	95	70	23	
		99	97	42	14	
		97	81	57	20	19
	0.2	109	68	87	27	
		104	71	66	21	24
	0.4	94	47	53	19	
		94	8	82	29	
		112	6	120	36	28
	0.5	Lethal				
		Lethal				
		Lethal				

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Study Performed at Litton Bionetics, Inc. (continued)						
-S9 (continued)						
Trial 3						
Dimethylsulfoxide						
		64	102	106	55	
		64	116	105	55	
		67	80	113	56	
		69	102	100	49	54
Ethyl methanesulfonate						
	250 μg/mL	32	25	721	751	
		33	40	670	672	
		25	19	692	941	788*
1,1,1-Trichloroethane						
	0.05	65	105	97	50	
		52	101	72	46	
		57	93	83	49	48
	0.1	53	95	75	47	
		42	75	86	69	
		71	105	105	49	55
	0.2	68	88	93	45	
		54	82	93	58	
		72	91	161	75	59
	0.3	66	75	104	52	
		86	92	134	52	
		74	76	116	53	52
	0.4	53	55	96	60	
		59	59	93	53	
		55	16	109	66	60
	0.5[g]	61	10	143	78	
		56	59	148	89	84
		Lethal				

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Study Performed at Litton Bionetics, Inc. (continued)						
+S9						
Trial 1						
Dimethylsulfoxide						
		103	109	108	35	
		82	86	106	43	
		92	105	100	36	38
Methylcholanthrene						
	5 μg/mL	21	2	211	343	
		22	2	326	494	419*
1,1,1-Trichloroethane						
	0.0078	91	56	91	33	
		99	107	159	54	44
	0.0156	102	111	154	50	
		79	86	148	63	57
	0.0313	88	73	176	66	
		102	99	176	58	62*
	0.0625	85	84	133	52	
		96	120	102	36	44
	0.125	106	75	133	42	
		103	110	176	57	49
	0.25	97	58	256	88	
		96	69	217	75	82*
	0.5[g]	70	5	172	82	
		Lethal				

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Study Performed at Litton Bionetics, Inc. (continued)						
+S9 (continued)						
Trial 2						
Dimethylsulfoxide						
		95	110	135	47	
		95	87	134	47	
		80	110	102	43	
		93	93	167	60	49
Methylcholanthrene						
	5 μg/mL	27	4	282	350	
		28	3	358	421	
		23	3	231	342	371*
1,1,1-Trichloroethane						
	0.025	87	75	241	92	
		100	96	193	65	
		87	86	127	48	68
	0.05	95	71	149	52	
		101	92	189	62	
		89	90	168	63	59
	0.1	118	92	224	63	
		98	78	196	66	
		104	73	242	77	69
	0.2	99	73	219	74	
		84	58	244	97	
		103	68	236	77	83*
	0.3	95	61	261	91	
		85	26	281	110	
		84	17	232	92	98*
	0.4	62	11	263	141	
		98	41	228	78	
		64	7	181	94	104*
	0.5	Lethal				
		Lethal				
		Lethal				

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Study Performed at Litton Bionetics, Inc. (continued)						
+S9 (continued)						
Trial 3						
Dimethylsulfoxide						
		86	102	100	39	
		102	90	92	30	
		89	101	70	26	
		95	107	100	35	33
Methylcholanthrene						
	5 μg/mL	31	5	269	289	
		28	5	269	324	
		29	4	298	341	318*
1,1,1-Trichloroethane						
	0.05	79	55	70	30	
		64	75	68	36	
		87	113	84	32	32
	0.1	70	76	78	37	
		82	66	64	26	
		86	83	71	28	30
	0.2	86	82	114	44	
		70	61	75	36	
		85	82	119	47	42
	0.3	74	58	67	30	
		75	65	62	28	
		64	66	70	37	32
	0.4	79	60	134	56	
		61	6	87	48	
		76	40	98	43	49
	0.5[g]	72	26	83	38	
		72	23	127	59	
		62	8	92	49	49*

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,1-Trichloroethane

Compound	Concentration (μL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Study Performed at Litton Bionetics, Inc. (continued)						
+S9 (continued)						
Trial 4						
Dimethylsulfoxide						
		100	115	52	17	
		104	93	81	26	
		98	105	69	23	
		104	86	86	28	24
Methylcholanthrene						
	2.5 μg/mL	79	54	491	207	
		88	56	593	225	
		96	56	638	221	218*
1,1,1-Trichloroethane						
	0.1	78	66	71	31	
		95	95	64	23	
		96	74	100	35	29
	0.2	98	78	99	34	
		95	82	85	30	
		94	65	85	30	31
	0.3	90	70	67	25	
		97	82	88	30	28
		Lethal				
	0.4	99	65	71	24	
		97	81	87	30	27
		Lethal				
	0.5[g]	113	84	60	18	
		96	79	84	29	
		101	86	98	33	26
		Lethal				

* Positive response ($P \leq 0.05$) versus the solvent control
[a] Doses were tested with two to four replicates; the average of the tests is presented in the table.
[b] Mutant fraction (MF) (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at $MF/10^6$ cells treated).
[c] The detailed protocol and these data are presented by Mitchell *et al*. (1988).
[d] Solvent control
[e] Positive control
[f] The detailed protocol and these data are presented by Myhr and Caspary (1988).
[g] Precipitation of 1,1,1-trichloroethane was noted at this concentration.

TABLE E3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 1,1,1-Trichloroethane[a]

Compound	Dose (μg/mL)	Total Cells Scored	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Trial 1								
Summary: Equivocal								
Dimethylsulfoxide[c]		50	1,047	430	0.41	8.6	26.0	
Mitomycin-C[d]	0.005	50	1,054	900	0.85	18.0	26.0	107.91
1,1,1-Trichloroethane	16	50	1,051	510	0.48	10.2	26.0	18.15
	50	50	1,051	425	0.40	8.5	26.0	−1.54
	160	50	1,048	443	0.42	8.9	26.0	2.92
	500	50	1,050	528	0.50	10.6	26.0	22.44*
					P=0.031[e]			
Trial 2								
Summary: Negative								
Dimethylsulfoxide		50	1,047	417	0.39	8.3	26.0	
Mitomycin-C	0.005	25	525	710	1.35	28.4	26.0	239.56
1,1,1-Trichloroethane	500	50	1,044	386	0.36	7.7	26.0	−7.17
	750	50	1,045	458	0.43	9.2	26.0	10.04
	1,000	50	1,043	459	0.44	9.2	26.0	10.49
					P=0.012			
+S9								
Summary: Equivocal								
Dimethylsulfoxide		50	1,045	482	0.46	9.6	26.0	
Cyclophosphamide[d]	1	25	523	487	0.93	19.5	26.0	101.88
1,1,1-Trichloroethane	16	50	1,043	448	0.42	9.0	26.0	−6.88
	50	50	1,041	494	0.47	9.9	26.0	2.88
	160	50	1,045	537	0.51	10.7	26.0	11.41
	500	50	1,045	537	0.51	10.7	26.0	11.41
					P=0.003			

* Positive response (≥20% increase over solvent control)
[a] Study was performed at Columbia University. The detailed protocol and these data are 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] Solvent control
[d] Positive control
[e] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose

TABLE E4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 1,1,1-Trichloroethane[a]

Compound	Dose (μg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
-S9					
Harvest time: 14 hours					
Summary: Positive					
Dimethylsulfoxide[b]					
		100	1	0.01	1.0
Mitomycin-C[c]					
	0.15	50	24	0.48	34.0
1,1,1-Trichloroethane					
	160	100	9	0.09	8.0*
	500	100	11	0.11	8.0*
	1,600	100	3	0.03	2.0
					P=0.379[d]
+S9					
Harvest time: 14 hours					
Summary: Negative					
Dimethylsulfoxide					
		100	4	0.04	4.0
Cyclophosphamide[c]					
	15	50	17	0.34	26.0
1,1,1-Trichloroethane					
	500	100	10	0.10	9.0
	1,600	100	14	0.14	11.0
	5,000	100	11	0.11	10.0
					P=0.054

* Positive response ($P \leq 0.05$) versus the solvent control
[a] Study was performed at Columbia University. The detailed protocol and these data are presented by Galloway *et al*. (1987).
[b] Solvent control
[c] Positive control
[d] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose

TABLE E5
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of 1,1,1-Trichloroethane in Feed for 13 Weeks[a]

Dose (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]
Males			
Untreated Control	5	0.80 ± 0.34	
Vehicle Control	5	0.90 ± 0.19	
5,000	5	1.10 ± 0.19	0.2519
10,000	5	1.20 ± 0.25	0.1789
20,000	5	1.50 ± 0.22	0.0520
40,000	5	1.50 ± 0.32	0.0520
80,000	5	1.80 ± 0.20	0.0115
		P=0.013[d]	
Females			
Untreated Control	5	0.60 ± 0.19	
Vehicle Control	5	0.80 ± 0.34	
5,000	5	0.80 ± 0.12	0.3815
10,000	5	1.20 ± 0.12	0.0827
20,000	5	1.70 ± 0.25	0.0055
40,000	5	1.20 ± 0.25	0.0827
80,000	5	1.20 ± 0.20	0.0827
		P=0.116	

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