NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicity Studies of

1,1,2,2-Tetrachloroethane (CAS No. 79-34-5) Administered in Microcapsules in Feed to F344/N Rats and $B6C3F_1$ Mice

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March 2004

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

These studies were supported in part by funds from the Comprehensive Environmental Response, Compensation, and Liability Act trust fund (Superfund) by an interagency agreement with the Agency for Toxic Substances and Disease Registry, U.S. Public Health Service.

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.

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

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PEER REVIEW

The draft report on the toxicity studies of 1,1,2,2-tetrachloroethane 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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SUMMARY

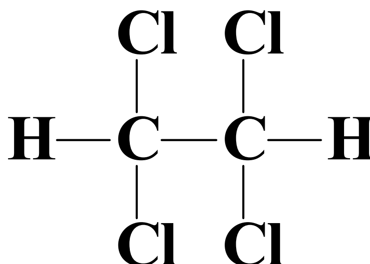
Background: 1,1,2,2-Tetrachloroethane was widely used in the production of solvents and pesticides. Its production ended in the 1990s, but it is a major component of waste sites. We studied the effects of 1,1,2,2-tetrachloroethane on male and female rats and mice to identify potential toxic hazards to humans.

Methods: Because 1,1,2,2-tetrachloroethane can evaporate easily, we enclosed it in starch microcapsules and placed them in the feed of rats and mice for 14 weeks. Male and female rats received up to 4,600 parts per million (ppm) 1,1,2,2-tetrachloroethane (equivalent to 0.46%) and mice received up to 9,100 ppm (0.91%). Control animals received empty starch microcapsules in their feed. Tissues from more than 40 sites were examined in all control and high-dose animals; tissues with lesions were examined in the lower exposure groups until no lesions were observed.

Results: Rats receiving 1,180 ppm or more 1,1,2,2-tetrachloroethane and mice receiving 2,300 ppm or more weighed less than the control animals. Male and female rats given 1,1,2,2-tetrachloroethane had pale and diseased livers and also had atrophy of the bone marrow and of the genital systems. Male and female mice given 1,1,2,2-tetrachloroethane had lesions of the liver and the bile duct.

Conclusion: We conclude that 1,1,2,2-tetrachloroethane at doses greater than 590 ppm in the feed was toxic to the liver of male and female rats. In mice, 1,1,2,2-tetrachloroethane was already known to cause cancer after long-term exposure. In these 14-week studies, 1,1,2,2-tetrachloroethane was toxic to the livers of male and female mice.

ABSTRACT



1,1,2,2-TETRACHLOROETHANE

CAS No. 79-34-5

Chemical Formula: $C_2H_2Cl_4$ Molecular Weight: 167.86

Synonyms: Acetylene tetrachloride; 1,1-dichloro-2,2-dichloroethane; *sym*-tetrachloroethane; TCE; 1,1,2,2-TCE; tetrachloroethane
Trade names: Acetosol, Bonoform, Boroform, Cellon

1,1,2,2-Tetrachloroethane is a solvent that was used in soil sterilization and as an ingredient in herbicides, insecticides, paints, varnishes, metal cleaners, and degreasers. Its production in the United States as an end-product ceased in the early 1990s. 1,1,2,2-Tetrachloroethane is currently used only as a chemical intermediate in the production of other chemicals. It was nominated for study because it was widely used and because it is found in hazardous waste sites and in surface water and groundwater. F344/N rats and B6C3F$_1$ mice were administered 1,1,2,2-tetrachloroethane (at least 99% pure) in microcapsules in the feed for 15 days or 14 weeks. Animals were evaluated for clinical pathology, reproductive system effects, and histopathology. Genetic toxicity studies were conducted *in vitro* in *Salmonella typhimurium*, L5178Y mouse lymphoma cells, and Chinese hamster ovary cells and *in vivo* in *Drosophila melanogaster* and mouse peripheral blood erythrocytes.

In the 15-day studies, groups of five male and five female rats and mice were fed diets containing 3,325, 6,650, 13,300, 26,600, or 53,200 ppm microencapsulated 1,1,2,2-tetrachloroethane. Additional groups of five male and five female rats and mice served as untreated controls, receiving feed without microcapsules, or as vehicle controls, receiving feed with empty microcapsules. Exposure concentrations of 3,325, 6,650, and 13,300 ppm resulted in average daily doses of 300, 400, and 500 mg 1,1,2,2-tetrachloroethane per kilogram body weight to male and female rats. All rats and mice exposed to 53,200 ppm, all rats and male mice exposed to 26,600 ppm, and two male mice exposed to 13,300 ppm died

or were killed moribund before the end of the studies. The mean body weights of all exposed groups of rats and mice with survivors were significantly less than those of the vehicle controls, and all of these groups except 3,325 ppm male rats and female mice lost weight during the studies. Clinical findings included thinness and ruffled fur in rats and mice; 53,200 ppm rats and 26,600 and 53,200 ppm male mice were lethargic, while male mice in the lower exposure groups and exposed female mice (except the 53,200 ppm group) were hyperactive. Thymus weights of rats exposed to 6,650 or 13,300 ppm and all exposed groups of female mice were significantly less than those of the vehicle controls. Liver weights of male rats in the 13,300 ppm group were also significantly less than those of the vehicle controls.

At necropsy, thin carcasses were noted in all exposed groups of male rats, in female rats exposed to 13,300 ppm or greater, in male mice exposed to 6,650 or 13,300 ppm, and in female mice exposed to 13,300 or 26,600 ppm. In rats, hepatodiaphragmatic nodules were noted grossly in one untreated control female, one female exposed to 6,650 ppm, one male and one female exposed to 13,300 ppm, and two males and one female exposed to 26,600 ppm; mild or moderate centrilobular degeneration was observed microscopically in the exposed rats with liver nodules. Pale or mottled livers were noted in all groups of exposed male and female mice and correlated microscopically with hepatocellular degeneration; the severity of hepatocellular degeneration increased with increasing exposure concentration.

In the 14-week studies, groups of 10 male and 10 female rats were fed 268, 589, 1,180, 2,300, or 4,600 ppm microencapsulated 1,1,2,2-tetrachloroethane, and groups of 10 male and 10 female mice received 589, 1,120, 2,300, 4,550, or 9,100 ppm, which resulted in average daily doses of 20 to 320 mg/kg for male and female rats, 100 to 1,360 mg/kg for male mice, and 80 to 1,400 mg/kg for female mice. Additional groups of 10 male and 10 female rats and mice served as untreated and vehicle controls. Groups of 10 male and 10 female special study rats designated for hematology and clinical chemistry analyses on study days 5 and 21 received the same exposure concentrations as the core study rats. All core study animals survived to the end of the studies. The mean body weights of male and female rats exposed to 1,180 ppm or greater and male and female mice exposed to 2,300 ppm or greater were generally significantly less than those of the vehicle controls. Male and female rats in the 4,600 ppm groups lost weight during the study. Clinical findings of toxicity included thinness and pallor in all rats in the 2,300 and 4,600 ppm groups and thinness in mice exposed to 2,300 ppm or greater. Results of the functional observation battery indicated no exposure-related findings of neurotoxicity in rats or mice.

Results of the hematology and clinical chemistry analyses indicated that exposure of rats and mice to 1,1,2,2-tetrachloroethane induced a hepatic effect, as demonstrated by increases in serum alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, and 5′-nucleotidase activities and total bile acid concentrations. Decreases in serum concentrations of total protein and cholesterol could also have been related to a liver effect or may have been related to the nutritional status of the animals. There was evidence indicating an effect on the circulating

erythroid mass, characterized by a minimal to mild microcytic nonresponsive anemia, in exposed rats. Minimal decreases in platelet and lymphocyte counts also occurred in exposed rats.

The thymus weights of female rats exposed to 4,600 ppm were significantly less than those of the vehicle controls. The liver weights of male and female rats increased with increasing exposure concentration up to 1,180 ppm; at higher exposure concentrations, absolute liver weights decreased along with decreasing body weights, although relative liver weights remained increased. The liver weights of male mice in the 1,120 and 2,300 ppm groups and females in all exposed groups were significantly greater than those of the untreated and vehicle controls. Kidney weights of male mice exposed to 2,300 ppm or greater were significantly less than those of the vehicle controls.

Thin carcasses, pale livers, and/or liver foci were noted grossly in exposed male and female rats and mice in the 14-week studies; additionally, exposed male rats had small testes and seminal vesicles and exposed female rats had small or thin uteri. Pale kidneys were observed in one male mouse in each of the 4,550 and 9,100 ppm groups. Microscopic lesions of minimal to moderate average severity were observed in the liver of exposed male and female rats, and splenic lesions were observed in male and female rats administered 1,180 ppm or greater. Male and female rats in the 4,600 ppm groups and females in the 2,300 ppm group also had atrophy of the bone metaphysis and bone marrow, prostate gland, preputial gland, seminal vesicle, testicular germinal epithelium, uterus, and clitoral gland. The incidence of cytoplasmic alteration of the ovarian interstitial cells was significantly increased in female rats in the 4,600 ppm group.

Liver hepatocyte hypertrophy and necrosis, focal pigmentation, and bile duct hyperplasia were observed in exposed male and female mice in the 14-week study. Males also had increased incidences of preputial gland atrophy.

Results of reproductive tissue evaluations in the 14-week studies indicated decreased left cauda epididymis, left epididymis, and left testis (mice) weights and epididymal spermatozoal motility in exposed male rats and mice relative to the vehicle controls. Female rats in the 2,300 ppm group spent more time in diestrus and less time in proestrus, estrus, and metestrus than did vehicle control females. The estrous cycle of female mice in the 9,100 ppm group was longer than that of the vehicle controls.

1,1,2,2-Tetrachloroethane was negative for induction of mutations in *S. typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 with and without S9 metabolic activation. It did not induce trifluorothymidine resistance in L5178Y mouse lymphoma cells with or without S9. In cytogenetic tests with cultured Chinese hamster ovary cells, 1,1,2,2-tetrachloroethane induced sister chromatid exchanges but not chromosomal aberrations in the presence and the absence of S9. No increases in the frequencies of sex-linked recessive lethal mutations were observed in germ cells of male *D. melanogaster* administered 1,1,2,2-tetrachloroethane via feeding or injection. Positive results were obtained

in the *in vivo* peripheral blood micronucleus test in mice in the 14-week feed study; significant increases in the frequencies of micronucleated normochromatic erythrocytes were observed in males and females.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

1,1,2,2-Tetrachloroethane, an aliphatic chlorinated hydrocarbon, is a colorless, volatile, corrosive liquid with a sickly sweet odor like chloroform, a melting point of −44° C, a boiling point of 146.5° C, a specific gravity of 1.58658 at 25° C, and a vapor pressure of 5 mm Hg at 21° C. It has a solubility of 2.96 g/L water at 25° C and is also soluble in organic solvents. 1,1,2,2-Tetrachloroethane is stable in the absence of air, moisture, and light and at high temperatures (*Merck Index*, 1996; ChemFinder.com, 2001; Syracuse Research Corporation, 2001).

PRODUCTION, USE, AND HUMAN EXPOSURE

1,1,2,2-Tetrachloroethane as an end-product was formerly produced in the United States only by the Specialty Materials Division of Eagle-Picher Industries in Lenexa, Kansas (SRI, 1988). By the late 1980s, this facility had been sold to the Vulcan Materials Company, and production was discontinued at the Kansas facilities (Montgomery and Welkom, 1990; SRI, 1993). Since the late 1980s, no production figures have been found. Approximately 440 million pounds (199.5 million kilograms) of 1,1,2,2-tetrachloroethane were produced in the United States in 1967 (Konietzko, 1984). Production declined markedly thereafter, falling to an estimated 34 million pounds (15.4 million kilograms) by 1974.

Commercial production of 1,1,2,2-tetrachloroethane as an end-product has apparently ceased in the United States. This parallels patterns in Canada, where the last plant to manufacture 1,1,2,2-tetrachloroethane as an end product ceased operations by 1985 (CEPA, 1993). At this time, any remaining production in the United States or Canada would involve 1,1,2,2-tetrachloroethane as a chemical intermediate, as a trace constituent in other chemicals, or as part of a waste stream in releases to the environment.

In the past, the major use for 1,1,2,2-tetrachloroethane was the production of trichloroethylene, tetrachloroethylene, and 1,2-dichloroethylene (Archer, 1979). It was also used as a solvent, in cleaning and degreasing metals, in paint removers, varnishes, and lacquers, in photographic films, and as an extractant for oils and fats (Hawley, 1981). Although at one time it could be used as an insecticide, fumigant, and weedkiller (Hawley, 1981), it currently is not registered for any of these purposes.

1,1,2,2-Tetrachloroethane can still appear as a chemical intermediate in the production of a variety of other common chemicals. Trace amounts of 1,1,2,2-tetrachloroethane may be introduced into the environment during the production of these chemicals, or it may appear as a minor impurity in the end products.

1,1,2,2-Tetrachloroethane is found in groundwater and surface water and is likely released from hazardous waste sites (Knox and Canter, 1996). Humans are exposed to 1,1,2,2-tetrachloroethane through ambient air and drinking water contamination. 1,1,2,2-Tetrachloroethane has been detected in the blood of the general population in the United States (Ashley *et al.*, 1994).

The Occupational Safety and Health Administration's permissible exposure limit is 35 mg/m^3 or 5 ppm 1,1,2,2-tetrachloroethane for an 8-hour, time-weighted average (TWA) in workplace air (NIOSH, 1997). The American Conference of Governmental Industrial Hygienists (2001) recommends a threshold limit value TWA of 6.9 mg/m^3 or 1 ppm for 1,1,2,2-tetrachloroethane.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

1,1,2,2-Tetrachloroethane is well absorbed via the dermal route in mice. It is metabolized by the hepatic P450 system (Torkelson and Rowe, 1981). The major metabolites detected were carbon dioxide (50%) in expired air and dichloroacetic acid (27%) and trichloroethanol (10%) in urine (Yllner, 1971). Minor metabolites were tri- and tetrachloroethylene in expired air. B6C3F_1 mice eliminated a larger amount of 1,1,2,2-tetrachloroethane as carbon dioxide than did Osborne-Mendel rats (Mitoma *et al.*, 1985).

1,1,2,2-Tetrachloroethane administered to male Wistar rats and BALB/c mice is bound covalently to DNA, RNA, and proteins of the liver, lung, kidney, and stomach (Colacci *et al.*, 1989). Eriksson and Brittebo (1991) reported that in C57BL mice injected intravenously with [^{14}C]-1,1,2,2-tetrachloroethane, a high and selective localization of bound metabolites occurred in the nasal olfactory mucosa; preferentially in the Bowman's gland; in epithelia of the trachea, bronchi, and bronchioli; in the squamous epithelia of the oral cavity, tongue, and esophagus; and in the liver, biliary bladder, inner zone of the adrenal cortex, and the interstitium of the testis.

Halpert (1982) proposed that 1,1,2,2-tetrachloroethane is activated by cytochrome P450 to dichloroacetyl chloride, which can bind covalently to various nucleophiles, causing lipid peroxidation and toxicity, or be hydrolyzed to dichloroacetic acid. The presence of the free radical intermediate ($CHCl_2CHCl$•) has been demonstrated in studies in rats (Tomasi *et al.*, 1984) and mice (Paolini *et al.*, 1992).

Humans

No information on the absorption, distribution, metabolism, or excretion of 1,1,2,2-tetrachloroethane by humans was found in a search of the literature.

TOXICITY

Experimental Animals

1,1,2,2-Tetrachloroethane is a powerful central nervous system depressant and is toxic to the liver, kidney, and lung in experimental animals. LD_{50} and LC_{50} values are given in Table 1. Hepatotoxic effects include release of the liver enzymes alanine aminotransferase and aspartate aminotransferase into the plasma; loss of hepatic cytochrome P450 and NADPH-cytochrome *c* reductase; reduction of phase II enzymes such as epoxide hydrolase, UDP-glucuronosyl transferase, and glutathione-*S*-transferase; reduction of heme biosynthesis and degradation; change in lipid levels; and elevated hepatic DNA synthesis (IARC, 1979; Bronzetti *et al.*, 1989; Paolini *et al.*, 1992; Cottalasso *et al.*, 1998). The effective dose in rats is 287 mg/kg or greater by oral administration (Cottalasso *et al.*, 1998); no toxic effects were observed at 1 mmol/kg (168 mg/kg) (Charbonneau *et al.*, 1991).

Transformation was observed in BALB/c 3T3 cells exposed to 1,1,2,2-tetrachloroethane (Colacci *et al.*, 1990, 1992). However, 1,1,2,2-tetrachloroethane did not promote transformation in BALB/c 3T3 cells exposed to *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine or 3-methylcholanthrene (Colacci *et al.*, 1996). 1,1,2,2-Tetrachloroethane was unable to transform BALB/c 3T3 cells in the absence of an exogenous metabolic activation system (Tu *et al.*, 1985).

TABLE 1
LD_{50} and LC_{50} Values for 1,1,2,2-Tetrachloroethane

Species	Route of Administration	LD_{50}/LC_{50}
Rat	Oral gavage	800 mg/kg
	Inhalation	1,000 ppm/4 hours
Mouse	Inhalation	4,500 mg/m^3/2 hours
	Intraperitoneal injection	30 mg/kg
	Intraperitoneal injection	820 mg/kg
	Subcutaneous injection	1,108 mg/kg

Humans

In humans, exposure to 1,1,2,2-tetrachloroethane via the inhalation, oral, or dermal route can cause fatigue, vomiting, dizziness, and liver damage. Hepatotoxocity, gastrointestinal disorders, and hematopoietic toxicity have been reported in humans following repeated exposure to 1,1,2,2-tetrachloroethane (IARC, 1979; Torkelson and Rowe, 1981). Workers exposed 1,1,2,2-tetrachloroethane had a moderately elevated (1.26 times) risk for leukemia, lymphoma, and cancer of the genital organs.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

No effects on the numbers of litters or pups of female rats exposed to 2 ppm 1,1,2,2-tetrachloroethane by inhalation for 325 days (Torkelson and Rowe, 1981). Injections of 700 mg/kg 1,1,2,2-tetrachloroethane in olive oil on day 9 of gestation or 400 mg/kg on days 7 through 14 were not embryotoxic to AB or DBA mice. Injections of 300 mg/kg 1,1,2,2-tetrachloroethane on days 1 through 14 were embryotoxic to DBA mice and induced low incidences of malformations: exencephaly, cleft palate, anophthalmia, and fused ribs and vertebrae (Schmidt, 1976). No conclusion on the reproductive or developmental toxicity of 1,1,2,2-tetrachloroethane can be drawn from these limited data.

Humans

No information on the reproductive or developmental toxicity of 1,1,2,2-tetrachloroethane in humans was found in a search of the literature.

CARCINOGENICITY

Experimental Animals

In carcinogenesis studies conducted by the National Cancer Institute (1978), 1,1,2,2-tetrachlorethane was administered in corn oil by gavage to groups of 50 male and 50 female Osborne-Mendel rats and B6C3F$_1$ mice at doses of 62 or 108 mg/kg (male rats), 43 or 76 mg/kg (female rats), and 142 or 282 mg/kg (mice) for 78 weeks followed by an observation period of 32 weeks for rats and 12 weeks for mice. Groups of 20 male and 20 female rats and mice served as vehicle controls, and additional groups of 20 males and 20 females served as untreated controls. At the end of the studies, hepatocellular carcinomas were observed in 2 of 16 (12.5%) male untreated control mice, 1 of 18 (5.5%) male vehicle control mice, 13 of 50 (26.0%) male mice administered 142 mg/kg, and 44 of 49 (89.8%) male mice administered 282 mg/kg. Hepatocellular carcinomas occurred in none of the untreated control or vehicle control female mice but did occur in 30 of 48 (62.5%) females in the 142 mg/kg group and 43 of 47 (91.5%) females in the 282 mg/kg

group. In the male rats, two hepatocellular carcinomas and one neoplastic nodule were observed in the 108 mg/kg group; no neoplasms were observed in female rats.

Metabolism and hepatic protein binding of 1,1,2,2-tetrachloroethane were greater in male $B6C3F_1$ mice than in male Osborne-Mendel rats. These biochemical parameters may be the bases for the different tumorigenic potency exhibited in rats and mice (Mitoma *et al.*, 1985). However, Colacci *et al.* (1987) studied macromolecular binding following intraperitoneal injection of 127 mCi/kg of [U-^{14}C]-1,1,2,2-tetrachloroethane in male Wistar rats and BALB/c mice and reported that binding to liver, kidney, and stomach DNA was stronger in mice than in rats; binding to lung DNA was similar in rats and mice. In contrast, binding to RNA and protein in the liver, kidney, lung, and stomach was greater in rats than in mice. 1,1,2,2-Tetrachloroethane behaved like a complete carcinogen with weak initiating activity and strong promoting activity in hepatectomized young adult male Osborne-Mendel rats, as measured by the induction of γ-glutamyl-transpeptidase (Milman *et al.*, 1988). In the BALB/c 3T3 cell transformation system, Colacci *et al.* (1993) reported that 1,1,2,2-tetrachlorethane induced transformation; the cells acquired a fully malignant phenotype. However, the chemical did not exert any promoting activity when tested in a two-stage system (Colacci *et al.*, 1996).

Humans

No information on epidemiology studies of 1,1,2,2-tetrachloroethane in humans was found in a search of the literature.

GENETIC TOXICITY

1,1,2,2-Tetrachloroethane has not been tested extensively for mutagenicity, but results of available studies provide limited evidence of activity *in vitro* and *in vivo*. 1,1,2,2-Tetrachloroethane was negative in *Salmonella typhimurium* gene mutation assays with and without S9 activation enzymes (Haworth *et al.*, 1983; Warner *et al.*, 1988). It did not induce chromosomal aberrations in cultured Chinese hamster ovary cells but did increase frequencies of sister chromatid exchanges with and without S9 (Galloway *et al.*, 1987). The positive results for sister chromatid exchanges were obtained at the same doses that yielded negative results in the chromosomal aberration tests. Although induction of sister chromatid exchanges is a measure of induced DNA damage, studies measuring induction of unscheduled DNA synthesis (repair-type synthesis) in rodent hepatocytes exposed to 1,1,2,2-tetrachloroethane *in vitro* (Williams *et al.*, 1989) or *in vivo* (Mirsalis *et al.*, 1989) gave negative results. Finally, no induction of sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* administered 1,1,2,2-tetrachloroethane by injection or feeding (Woodruff *et al.*, 1985).

STUDY RATIONALE

The use of 1,1,2,2-tetrachloroethane as an intermediate in the production of large volumes of halogenated hydrocarbon solvents suggested that substantial quantities of 1,1,2,2-tetrachlorethane have been disposed of in hazardous waste sites. 1,1,2,2-Tetrachloroethane has been found in at least 273 of the 1,430 National Priority List sites identified by the Environmental Protection Agency (ATSDR, 1997). 1,1,2,2-Tetrachloroethane has been on the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Priority List of Hazardous Substances since 1987 (ATSDR, 1996). The CERCLA Priority List of Hazardous Substances is the list of the 275 most frequently reported compounds at hazardous waste sites included on the National Priorities List (ATSDR, 2001). The Hazardous Waste Information Evaluation Subcommittee recommended to the ATSDR that further toxicity studies be done on 1,1,2,2-tetrachloroethane. The primary route of exposure to 1,1,2,2-tetrachlorethane is by drinking water. However, because the solubility of 1,1,2,2-tetrachlorethane in water is limited and high concentrations of the chemical may not be palatable, the ATSDR recommended that 1,1,2,2-tetrachloroethane be administered in feed via microencapsulation.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 1,1,2,2-TETRACHLOROETHANE

1,1,2,2-Tetrachloroethane was obtained from Eastman Kodak Company (Rochester, NY) in one lot (B17) for use in the 15-day and 14-week studies. The microencapsulation of the chemical was performed by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the microcapsules were assigned a separate lot number (335-2A). Identity, purity, stability, and water content analyses of the neat and microencapsulated chemical were conducted by the analytical chemistry laboratory and the study laboratories. Reports on analyses performed in support of the 1,1,2,2-tetrachloroethane studies are on file at the National Institute of Environmental Health Sciences.

Analyses of Neat Chemical

The chemical, a clear, colorless liquid, was identified as 1,1,2,2-tetrachloroethane by the analytical chemistry laboratory using infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy; identity was confirmed by the study laboratories using infrared spectroscopy. All spectra were consistent with the literature spectra (*Sadtler Standard Spectra*, 1970) and with the structure of 1,1,2,2-tetrachloroethane. The infrared and nuclear magnetic resonance spectra are presented in Figures F1 and F2.

The purity of lot B17 was determined by elemental analyses and gas chromatography (GC). Additional GC analyses with mass spectroscopy (GC/MS) were performed to determine whether selected chlorinated impurities were present and to quantify any impurities that were detected. The 14-week study laboratory analyzed purity using GC. Elemental analyses for carbon, hydrogen, and chlorine were in agreement with the theoretical values for 1,1,2,2-tetrachloroethane. GC by two systems indicated one major peak and one impurity with an area of 0.15% or 0.13% relative to the major peak area. Trichloroethylene (393 ± 35 ppm) and tetrachloroethylene (13 ± 1 ppm) were identified as impurities by GC/MS. Additionally, chloroform, *cis*-1,2-dichloroethylene, and *trans*-1,2-dichloroethylene were tentatively identified as impurities at concentrations less than 1 ppm. The overall purity of lot B17 was determined by the analytical chemistry laboratory to be greater than 99%. Using GC, the study laboratory confirmed that the purity was 99% or greater; one impurity with an area greater than 0.1% of the total peak area and four minor impurities were detected. Karl Fischer titration indicated 0.014% ± 0.007% water.

Based on the manufacturer's recommendations, the bulk chemical was stored frozen.

Microcapsule Formulation and Analyses

Microcapsules loaded with neat 1,1,2,2-tetrachloroethane and placebos (empty microcapsules) were prepared by the analytical chemistry laboratory with a proprietary process using food-grade, modified corn starch and reagent-grade sucrose (80:20) to produce dry microspheres; the outer surfaces of the microcapsules were dusted with food-grade, hydrophobic, modified corn starch. Following microencapsulation, the analytical chemistry laboratory tested the chemical for conformance to specifications. The microcapsules were examined microscopically for appearance. Conformance to particle size specifications (with no more than 1% of particles having diameters greater than 420 µm) was determined by passing placebo and loaded microcapsules through U.S. standard sieves (Numbers 30, 40, 60, 80, 100, and 120). The chemical loads of freshly prepared microcapsules and of microcapsules stored under a variety of conditions were determined with GC. Comparisons of the impurity profiles of neat and microencapsulated 1,1,2,2-tetrachloroethane and 4- and 20-month stability studies were also performed with GC.

Microscopic examination of the microcapsules revealed no unusual characteristics. Loaded microcapsules were slightly outside the size specification, with 1.1% having diameters greater than 420 µm; this was not expected to have a significant effect on the studies. The placebo particles were within the size specifications. The mean 1,1,2,2-tetrachloroethane load was 54.0% ± 0.3%. Microcapsules exposed to animal room conditions (50% relative humidity, 25° C) in open dishes retained 98.8% of their chemical load by weight after 28 days; additional samples similarly exposed after seven freeze-thaw cycles and samples stored in sealed bottles at 5° C retained 99.1% of their initial chemical load after 28 days. Comparison of impurity profiles indicated that no impurities or significant changes in the impurity profile were introduced by microencapsulation. Results of the 4- and 20-month shelf life studies indicated that microcapsules retained greater than 98% of their chemical load when stored in sealed containers at room temperature for 4 months and greater than 99% when stored at 5° C for 20 months.

The study laboratories confirmed the identity of the microcapsules with infrared spectroscopy and analyzed the chemical load of the microcapsules using GC. GC analyses indicated a chemical load of 53.2% ± 0.8% at the beginning of the 15-day studies and 52.4% at the beginning of the 14-week studies. To ensure stability, the microcapsules were stored at room temperature, protected from light, during the 15-day studies and at approximately 5° C, protected from light and moisture, during the 14-week studies. The study laboratories monitored the stability of the microencapsulated chemical during the studies with GC; no loss of 1,1,2,2-tetrachloroethane was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared once during the 15-day studies and at least every 3 weeks during the 14-week studies by mixing microencapsulated 1,1,2,2-tetrachloroethane with feed (Table F2). In the 15-day studies, placebo

and/or loaded microcapsules were combined with feed to a concentration of 10% microcapsules; in the 14-week studies, the concentrations of microcapsules in feed were 0.86% for rats and 1.7% for mice. A premix was prepared by hand and then blended with additional feed in a twin-shell blender for 15 minutes. The dose formulations were kneaded and mixed manually and then mixed for an additional 15 minutes in the blender. In the 15-day studies, dose formulations were stored in plastic bags, protected from light, at room temperature for up to 3 weeks; dose formulations for the 14-week studies were stored in plastic bags, protected from light and moisture, at 5° C for up to 4 weeks.

Homogeneity and stability studies of a dose formulation containing 0.5% microencapsulated 1,1,2,2-tetrachloroethane were performed using GC. Homogeneity was confirmed, and stability studies indicated that samples were stable for 33 days when stored at 5° C. Samples stored at room temperature for 4 days, open to air and light, or for 33 days, protected from air and light, had small but significant losses of 1,1,2,2-tetrachloroethane.

The study laboratories performed homogeneity studies of the 3,325 and 53,200 ppm dose formulations for the 15-day studies and the 268, 589, 4,600, and 9,100 ppm dose formulations for the 14-week studies, as well as stability studies of the 268 ppm dose formulation, using GC. Homogeneity was confirmed, and stability was confirmed for 28 days for dose formulations stored at room temperature or at approximately 5° C and for 1 week for dose formulations stored at room temperature under simulated animal room conditions, open to air and light.

During the 14-week studies, periodic analyses of the dose formulations were conducted by the study laboratory using GC. The dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples of these dose formulations were also analyzed (Table F3). Of the dose formulations analyzed, 13 of 15 for rats and 12 of 15 for mice were within 10% of the target concentrations, with no value greater than 111% of the target concentration. Five dose formulations with concentrations that were only slightly outside the 10% criterion were considered suitable for use in the studies. For the animal room samples, 12 of 15 for rats and 8 of 15 for mice were within 10% of the target concentrations; these results were attributed to environmental degradation of the microcapsule matrix, ability of the animals to separate feed from microcapsules, and/or analytical variation.

15-Day Studies

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, rats and mice were 31 days old. Rats were quarantined for 12 days, and mice were quarantined for 13 days; the animals were 43 (rats) or 44 (mice) days old on the first day of the studies. Groups of five male and five female rats and mice were fed diets containing 3,325, 6,650, 13,300, 26,600, or 53,200 ppm microencapsulated 1,1,2,2-tetrachloroethane. The highest exposure concentration was based on published oral LD$_{50}$ values. The animals were thought to be able to

tolerate a higher exposure concentration in feed than by gavage. Additional groups of five male and five female rats and mice received untreated feed (untreated controls) or feed containing placebo microcapsules (vehicle controls). Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded twice daily and animals were weighed initially, on day 8, and at the end of the studies. Feed consumption was recorded on day 8, day 11 (rats in the 26,600 and 53,000 ppm groups), and at the end of the studies. At the beginning of the studies, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Details of the study design and animal maintenance are summarized in Table 2.

At the end of the studies, necropsies were performed on all rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. All gross lesions observed at necropsy were examined microscopically.

14-WEEK STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms. On receipt, the rats and mice were 5 weeks old. Animals were quarantined for 14 or 15 days (rats) or 12 or 13 days (mice) and were 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. Blood samples were collected from five male and five female untreated control rats and four male and five female sentinel mice at the end of the 14-week studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results were negative.

Groups of 10 male and 10 female animals were fed diets containing 268, 589, 1,180, 2,300, or 4,600 ppm (rats) or 589, 1,120, 2,300, 4,550, or 9,100 ppm (mice) microencapsulated 1,1,2,2-tetrachloroethane. Additional groups of 10 male and 10 female rats and mice received untreated feed (untreated controls) or feed containing placebo microcapsules (vehicle controls). Groups of 10 male and 10 female special study rats designated for hematology and clinical chemistry analyses on study days 5 and 21 received the same exposure concentrations as the core study rats. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Feed consumption was recorded weekly (rats and male mice) or twice weekly (female mice) by cage. Clinical findings were recorded and the animals were weighed initially, weekly, and at the end of the studies. Functional observation batteries were performed on core study untreated and vehicle control rats and mice; rats in the 268, 589, and 1,180 ppm groups; and mice in the 1,120, 2,300, and 4,550 ppm groups during weeks 4 and 13. Details of the study design and animal maintenance are summarized in Table 2.

On days 5 and 21, blood was collected from the retroorbital sinus of groups of 10 male and 10 female special study rats for hematology and clinical chemistry analyses. At the end of the 14-week studies, blood was collected from the retroorbital sinus of core study rats for hematology and clinical chemistry analyses and from all mice for clinical chemistry analyses. Blood for hematology determinations was placed in tubes containing EDTA. For clinical chemistry analyses, samples were collected in tubes with no anticoagulant. Manual hematocrit determinations were performed using an Adams Microhematocrit centrifuge (Model CT2900, Clay Adams, Sparks, MD). All other hematology parameters were measured by a Serono-Baker 9000 automated cell counter (Baker Instruments, Allentown, PA) using reagents supplied by the manufacturer. Leukocyte differential counts and erythrocyte, total leukocyte, nucleated erythrocyte, and platelet counts were determined microscopically from blood films stained with modified Wright's stain on an Ames Hema-Tek® Slide Stainer (Miles Laboratory, Ames Division, Elkhart, IN). Reticulocyte counts were determined from slides prepared with blood stained with new methylene blue (Sigma Chemical Company, St. Louis, MO) and counted microscopically using a Miller disc. Clinical chemistry parameters were measured with a Hitachi® 717 chemistry analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN); all reagents were obtained from the manufacturer with the exception of the reagents for sorbitol dehydrogenase and total bile acid determinations, which were obtained from Sigma Chemical Company. The parameters measured are listed in Table 2.

At the end of the 14-week core studies, samples were collected for sperm motility and vaginal cytology evaluations from untreated and vehicle control rats and mice, rats exposed to 589, 1,180, or 2,300 ppm, and mice exposed to 1,120, 4,550, or 9,100 ppm. The parameters evaluated are listed in Table 2. Methods used were those described in the NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1991). For 12 consecutive days prior to sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

At the end of the studies, necropsies were performed on core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all control animals, rats in the 4,600 ppm groups, and mice in the 9,100 ppm groups. Table 2 lists the tissues and organs routinely examined.

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 2
Experimental Design and Materials and Methods in the Feed Studies of 1,1,2,2-Tetrachloroethane

15-Day Studies	14-Week Studies
Study Laboratory	
TSI Mason Laboratories (Worcester, MA)	Microbiological Associates, Inc. (Bethesda, MD)
Strain and Species	
Rats: F344/N Mice: B6C3F$_1$	Rats: F344/N Mice: B6C3F$_1$
Animal Source	
Taconic Farms (Germantown, NY)	Taconic Farms (Germantown, NY)
Time Held Before Studies	
Rats: 12 days Mice: 13 days	Rats: 14 days (males) or 15 days (females) Mice: 13 days (males) or 12 days (females)
Average Age When Studies Began	
Rats: 43 days Mice: 44 days	7 weeks
Date of First Exposure	
Rats: February 25, 1992 Mice: February 26, 1992	Rats: June 3 (males) or 4 (females), 1993 Mice: June 2 (males) or 1 (females), 1993
Duration of Exposure	
15 days	14 weeks
Date of Last Exposure	
Rats: March 10, 1992 Mice: March 11, 1992	Rats: September 2 (males) or 3 (females), 1993 Mice: September 1 (males) or August 31 (females), 1993
Necropsy Dates	
Rats: March 10, 1992 Mice: March 11, 1992	Rats: September 2 (males) or 3 (females), 1993 Mice: September 1 (males) or August 31 (females), 1993
Average Age at Necropsy	
Rats: 57 days Mice: 58 days	20 weeks
Size of Study Groups	
5 males and 5 females	10 males and 10 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 15-day studies
Animals per Cage	
Rats: 5 Mice: 1 (males) or 5 (females)	Same as 15-day studies
Method of Animal Identification	
Tail tattoo	Tail tattoo

TABLE 2
Experimental Design and Materials and Methods in the Feed Studies of 1,1,2,2-Tetrachloroethane

15-Day Studies	14-Week Studies
Diet	
NIH-07 open formula mash diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*
Water	
Tap water (Worcester municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Tap water (Washington Suburban Sanitary Commission, Potomac Plant, MD) via automatic watering system, available *ad libitum*
Cages	
Polycarbonate (Lab Products, Inc., Rochelle Park, NJ), changed twice per week	Polycarbonate, changed twice weekly (rats and female mice) or once weekly (male mice)
Bedding	
Hardwood chips (P.J. Murphy Forest Products, Montville, NJ), changed twice weekly	Same as 15-day studies except rats and female mice were changed twice weekly, male mice weekly
Racks	
Stainless steel (Lab Products, Inc., Rochelle Park, NJ), changed every 2 weeks	Stainless steel, changed every 2 weeks
Animal Room Environment	
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Exposure Concentrations	
0, 3,325, 6,650, 13,300, 26,600, or 53,200 ppm, microencapsulated in feed	Rats: 0, 268, 589, 1,180, 2,300, or 4,600 ppm, microencapsulated in feed Mice: 0, 589, 1,120, 2,300, 4,550, or 9,100 ppm, microencapsulated in feed
Type and Frequency of Observation	
Observed and clinical findings recorded twice daily; animals were weighed initially, on day 8, and at the end of the studies. Feed consumption was recorded on day 8, day 11 (rats in the 26,600 and 53,200 ppm groups), and at the end of the studies.	Observed twice daily; animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies. Feed consumption was recorded weekly (rats and male mice) or twice weekly (female mice) by cage.
Method of Sacrifice	
Carbon dioxide asphyxiation	Same as 15-day studies
Necropsy	
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.

TABLE 2
Experimental Design and Materials and Methods in the Feed Studies of 1,1,2,2-Tetrachloroethane

15-Day Studies	14-Week Studies
Clinical Pathology	
None	Blood was collected from the retroorbital sinus of special study rats on days 5 and 21 and from core study rats surviving to the end of the studies for hematology and clinical chemistry. Blood was collected from the retroorbital sinus of all mice surviving to the end of the study for clinical chemistry. ***Hematology:*** automated and manual hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count; and total leukocyte count and differentials ***Clinical chemistry:*** creatinine, total protein, albumin, cholesterol, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, 5′-nucleotidase, and bile acids
Histopathology	
All gross lesions observed at necropsy were microscopically examined.	Complete histopathologic examinations were performed on core study untreated and vehicle control animals, rats in the 4,600 ppm groups, and mice in the 9,100 ppm groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular, mesenteric), mammary gland with adjacent skin, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The bone with marrow, clitoral gland, liver, ovary, prostate gland, spleen, testis with epididymis and seminal vesicle, and uterus were also examined in rats in the lower exposure groups. The liver, spleen, and thymus of male and female mice, the preputial gland of male mice, and the lung of female mice were examined in the lower exposure groups.
Functional Observation Battery	
None	Functional observation batteries were performed on core study untreated and vehicle control rats and mice; rats in the 268, 589, and 1,180 ppm groups; and mice in the 1,120, 2,300, and 4,550 ppm groups during weeks 4 and 13. The following parameters were observed: general behavior, body position, convulsions, activity level, gait, coordination, compulsive biting or licking, head-flick, head searching, backward walking, self-mutilation, circling, lacrimation or chromodacryorrhea, piloerection, pupillary dilation or constriction, salivation, diarrhea, tremors, unusual respiration, excessive or diminished urination, and vocalization.

Table 2
Experimental Design and Materials and Methods in the Feed Studies of 1,1,2,2-Tetrachloroethane

15-Day Studies	14-Week Studies
Sperm Motility and Vaginal Cytology	
None	At the end of the studies, sperm samples were collected from core study untreated and vehicle control rats and mice; rats in the 589, 1,180, and 2,300 ppm groups; and mice in the 1,120, 4,550, and 9,100 ppm groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda epididymis, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from all core study untreated and vehicle control rats and mice; rats in the 589, 1,180, and 2,300 ppm groups; and mice in the 1,120, 4,550, and 9,100 ppm groups for vaginal cytology evaluations. The percentage of time spent in the various estrous stages and estrous cycle length were evaluated.

Statistical Methods

Calculation and Analysis of Lesion Incidences

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

Analysis of Continuous Variables

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

analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations. For functional observation battery parameters, exposed groups were compared to the controls with a chi-square test.

QUALITY ASSURANCE METHODS

The 14-week studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Unit of Microbiological Associates, Inc., performed audits and inspections of protocols, procedures, data, and reports throughout the course of the 14-week studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed at Case Western Reserve University as reported by Haworth *et al.* (1983); the protocol was modified for the Inveresk Research International Study. 1,1,2,2-Tetrachloroethane was sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat, Syrian hamster, or B6C3F$_1$ mouse liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of 1,1,2,2-tetrachloroethane. The high dose was limited by toxicity. All trials were repeated.

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

Mouse Lymphoma Mutagenicity Test Protocol

The experimental protocol is presented in detail by Myhr *et al.* (1985). 1,1,2,2-Tetrachloroethane was supplied as a coded aliquot by Radian Corporation. The high dose of 1,1,2,2-tetrachloroethane was determined by 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 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, horse serum content was increased and Noble agar was added.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 mL medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with 1,1,2,2-tetrachloroethane continued for 4 hours, at which time the medium plus 1,1,2,2-tetrachloroethane 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 test was initially performed without S9. Because a clearly positive response was not obtained, the test was repeated using freshly prepared S9 from the livers of either Aroclor 1254-induced or uninduced male F344/N rats.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented by Caspary *et al.* (1988). All data were evaluated statistically for trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for 1,1,2,2-tetrachloroethane to be considered positive, i.e., capable of inducing TFT resistance. A single significant response led to a call of "questionable," 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). 1,1,2,2-Tetrachloroethane 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 six concentrations of 1,1,2,2-tetrachloroethane; the high dose was limited by toxicity. A single flask per dose was used.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 25.8 hours with 1,1,2,2-tetrachloroethane in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 25.8 hours, the medium containing 1,1,2,2-tetrachloroethane 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 1,1,2,2-tetrachloroethane, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no 1,1,2,2-tetrachloroethane. Incubation proceeded for an additional 25.5 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/cell from each dose level. Because significant chemical-induced cell cycle delay was seen at the highest concentration tested in the absence of S9, incubation time for that culture was lengthened to ensure a sufficient number of scorable (second-division metaphase) cells.

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 1,1,2,2-tetrachloroethane for 19.5 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with 1,1,2,2-tetrachloroethane and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 18.5 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; because cell cycle delay was anticipated, the incubation period was extended beyond the usual time of 10 to 12 hours.

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

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a

statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose point 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.

Drosophila melanogaster Test Protocol

The assay for induction of sex-linked recessive lethal (SLRL) mutations was performed with adult flies as described by Woodruff *et al.* (1985). 1,1,2,2-Tetrachloroethane was supplied as a coded aliquot from Radian Corporation. 1,1,2,2-Tetrachloroethane was assayed by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no response was obtained, it was retested by injection into adult males.

To administer 1,1,2,2-tetrachloroethane by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament, and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 µL) to slightly distend the abdomen of the fly, or automatically, by attaching the pipette to a microinjector that delivered a calibrated volume. Flies were anaesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of 1,1,2,2-tetrachloroethane at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. Canton-S males were allowed to feed for 72 hours on a solution of 1,1,2,2-tetrachloroethane in 5% sucrose dissolved in 10% ethanol. In the injection experiments, 24- to 72-hour-old Canton-S males were treated with 1,1,2,2-tetrachloroethane dissolved in 10% ethanol and diluted with 0.7% sodium chloride and allowed to recover for 24 hours. A concurrent saline control group was also included. Treated males were mated to three *Basc* females for 3 days and were given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings were treated at successively earlier postmeiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event, and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution). If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

SLRL data were analyzed by simultaneous comparison with the concurrent and historical controls (Mason *et al.*, 1992) using a normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if the P value was less than or equal to 0.01 and the mutation frequency in the tested group was greater than 0.10%, or if the P value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or if the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P value was greater than or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 14-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 and coded. 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 using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposure group and the vehicle 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 exposure 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). Results of the 14-week studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among

laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The results presented in the Abstract of this Toxicity Study Report represent a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

RATS

15-DAY STUDY

All rats exposed to 26,600 or 53,200 ppm were killed moribund on day 11; all other animals survived to the end of the study (Table 3). The final mean body weights and body weight gains of males and females in all exposed groups with survivors were significantly less than those of the vehicle controls; males and females in the 6,650 and 13,300 ppm groups and females in the 3,325 ppm group lost weight during the study (Table 3). Feed consumption decreased with increasing exposure concentration. Exposure concentrations of 3,325, 6,650, and 13,300 ppm resulted in average daily doses of 300, 400, and 500 mg 1,1,2,2-tetrachloroethane per kilogram body weight to males and females. Males exposed to 13,300 ppm or greater and females exposed to 6,650 ppm or greater were thin and had ruffled fur. All males and females in the 53,200 ppm groups were lethargic.

Absolute and relative thymus weights of males and females exposed to 6,650 or 13,300 ppm and absolute liver weights of males in the 13,300 ppm group were less than those of the vehicle controls (Table C1). Relative kidney weights were generally significantly increased in exposed groups of rats even though absolute kidney weights in these groups were significantly decreased. Other differences in organ weights generally reflected body weight changes.

At necropsy, thin carcasses were observed in all exposed groups of males and in females exposed to 13,300 ppm or greater. Hepatodiaphragmatic nodules were noted grossly in one untreated control female, one female exposed to 6,650 ppm, one male and one female exposed to 13,300 ppm, and two males and one female exposed to 26,600 ppm. Focal areas of alopecia occurred on the skin of two males and all females in the 53,200 ppm group and in four females in the 13,300 ppm group; microscopically, these lesions correlated with minimal to moderate acanthosis in the four affected females in the 13,300 ppm group and in four females in the 53,200 ppm group, but not in the males. Mild or moderate centrilobular degeneration was observed microscopically in the livers of the exposed males and females that were noted grossly to have liver nodules; degeneration was considered to be related to exposure.

Based on the early deaths of rats exposed to 26,600 or 53,200 ppm and the decreased body weight gains of rats exposed to 3,325 ppm or greater, the exposure concentrations selected for rats in the 14-week study were 268, 589, 1,180, 2,300, and 4,600 ppm.

TABLE 3
Survival, Body Weights, and Feed Consumption of Rats in the 15-Day Feed Study of 1,1,2,2-Tetrachloroethane

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Vehicle Controls (%)	Feed Consumption[c]
Male						
Untreated Control	5/5	149 ± 5	232 ± 5	83 ± 1		18.5
Vehicle Control	5/5	153 ± 5	235 ± 5	82 ± 2		18.8
3,325	5/5	152 ± 6	168 ± 8**	16 ± 5**	72	14.9
6,650	5/5	150 ± 7	125 ± 3**	−26 ± 4**	53	8.0
13,300	5/5	153 ± 5	108 ± 2**	−45 ± 4**	46	4.9
26,600	0/5[d]	150 ± 7	—	—	—	2.4
53,200	0/5[d]	151 ± 6	—	—	—	1.4
Female						
Untreated Control	5/5	117 ± 2	152 ± 3	35 ± 3		13.0
Vehicle Control	5/5	117 ± 3	152 ± 3	35 ± 2		12.8
3,325	5/5	118 ± 2	114 ± 2**	−4 ± 2**	75	9.7
6,650	5/5	116 ± 2	95 ± 1**	−21 ± 2**	63	6.2
13,300	5/5	117 ± 2	81 ± 2**	−36 ± 1**	53	3.7
26,600	0/5[d]	116 ± 3	—	—	—	1.7
53,200	0/5[d]	115 ± 2	—	—	—	1.2

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Number of animals surviving at 15 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. No final mean body weights or weight changes were calculated for groups with 100% mortality.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.
[d] Day of deaths: 11

14-WEEK STUDY

All rats survived to the end of the study (Table 4). The final mean body weights and body weight gains of males and females exposed to 1,180 ppm or greater were significantly less than those of the vehicle controls; the mean body weight gain of females in the 589 ppm group was also significantly less than that of the vehicle controls (Table 4 and Figure 1). Males and females in the 4,600 ppm groups lost weight during the study. Feed consumption generally decreased with increasing exposure concentration. Exposure concentrations of 268, 589, 1,180, 2,300, and 4,600 ppm resulted in average daily doses of 20, 40, 80, 170, and 320 mg/kg for males and females. Clinical findings of toxicity included thinness and pallor in all rats in the 2,300 and 4,600 ppm groups. Results of the functional observation battery indicated no exposure-related findings of neurotoxicity.

TABLE 4
Survival, Body Weights, and Feed Consumption of Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Vehicle Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 2	Week 13
Male							
Untreated Control	10/10	134 ± 3	357 ± 10	222 ± 8			
Vehicle Control	10/10	135 ± 3	366 ± 5	231 ± 4			
268	10/10	133 ± 3	354 ± 9	221 ± 7	97	15.3	16.6
589	10/10	135 ± 4	353 ± 6	218 ± 4	96	14.6	16.3
1,180	10/10	133 ± 2	341 ± 6*	207 ± 6**	93	14.4	17.2
2,300	10/10	133 ± 3	259 ± 9**	126 ± 6**	71	13.7	14.5
4,600	10/10	133 ± 3	127 ± 5**	−6 ± 5**	35	10.1	8.0
Female							
Untreated Control	10/10	108 ± 3	193 ± 6	85 ± 3			
Vehicle Control	10/10	108 ± 2	195 ± 4	87 ± 3			
268	10/10	110 ± 2	192 ± 4	82 ± 2	98	10.6	10.1
589	10/10	110 ± 2	189 ± 2	79 ± 2*	97	10.8	9.9
1,180	10/10	111 ± 2	177 ± 2**	66 ± 2**	91	10.3	10.3
2,300	10/10	108 ± 3	139 ± 4**	31 ± 3**	71	10.3	9.0
4,600	10/10	110 ± 2	85 ± 3**	−25 ± 3**	44	7.5	5.8

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.

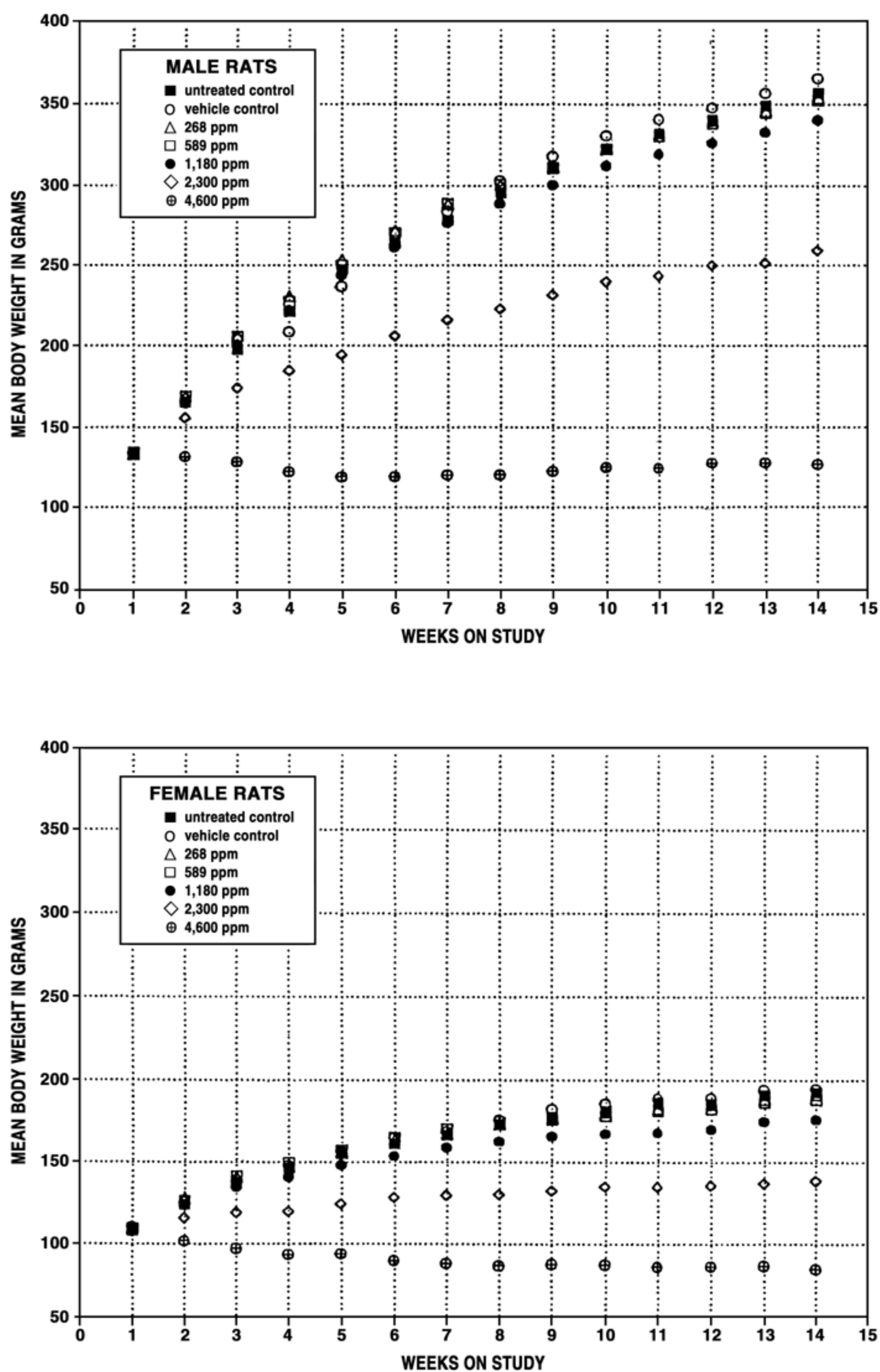


Figure 1
Body Weights of Male and Female Rats Exposed to 1,1,2,2-Tetrachloroethane in Feed for 14 Weeks

Hematology and clinical chemistry data for rats are given in Tables 5 and B1. On day 5, a minimal exposure concentration-related erythrocytosis, evidenced by increases in automated and manual hematocrit values, hemoglobin concentrations, and erythrocyte counts, occurred in 4,600 ppm males and females and 2,300 ppm females. This erythrocytosis persisted to day 21 and was accompanied by decreased reticulocyte counts. At week 14, the erythrocytosis disappeared and was replaced by minimal to mild, exposure concentration-related anemia, evidenced by decreased hematocrit values and hemoglobin concentrations in rats exposed to 589 ppm or greater. In an apparently inconsistent response, the week 14 decrease in hematocrit values and hemoglobin concentrations was not accompanied by changes in erythrocyte counts. Evidence suggesting a treatment-related erythropoietic effect included decreases in mean cell volumes, mean cell hemoglobin values, and mean cell hemoglobin concentrations predominantly in males and females exposed to 2,300 ppm or greater and in 1,180 ppm females at various time points. The minimal to mild, exposure concentration-related decreases in mean cell volumes and mean cell hemoglobin values in males and females in these groups suggest that the circulating erythrocytes were smaller (microcytic) than what would be expected. Thus, regardless of the generally unchanged erythrocyte counts at week 14, the consequence of smaller circulating erythrocytes was an overall decrease in the erythron. At week 14, there were no changes in reticulocyte counts, suggesting that there was no erythropoietic response to the anemia.

On day 5, the platelet count in 4,600 ppm males was minimally decreased compared to the vehicle controls. This decrease became more pronounced and, by day 21, occurred in males and females exposed to 1,180 ppm or greater and in 589 ppm females. By week 14, the decreases in platelet counts had ameliorated and occurred only in females in the 2,300 and 4,600 ppm groups. At week 14, decreased leukocyte counts, characterized by decreases in lymphocyte counts, occurred in males and females exposed to 2,300 or 4,600 ppm. This lymphopenia would be consistent with a physiological stress/steroid-induced response.

Several treatment-related alterations in clinical chemistry parameters occurred in males and females (Tables 5 and B1). At all time points, alanine aminotransferase and sorbitol dehydrogenase activities were minimally to markedly increased in exposed males and females. The magnitude of these alterations increased with time, and the increases were consistent in 1,180 ppm males and 2,300 and 4,600 ppm males and females; these changes would suggest hepatocellular injury or leakage. The concentrations of total bile acids, a marker of cholestasis or altered hepatic function, generally were mildly to markedly increased in rats exposed to 2,300 and 4,600 ppm at all time points. The magnitude of these changes was exposure concentration related and was greatest on day 21. The activities of alkaline phosphatase and 5′-nucleotidase, other markers of cholestasis, were minimally to moderately increased in 2,300 and 4,600 ppm males and females on days 5 and 21; at week 14, the cholestasis effect in these groups was demonstrated by the continued elevation of alkaline phosphatase activities.

TABLE 5
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Male						
n						
Day 5	10	10	10	10	10	10
Day 21	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Hematology						
Automated hematocrit (%)						
Day 5	38.8 ± 0.4	37.8 ± 1.1	39.4 ± 0.5	39.3 ± 0.5	40.3 ± 0.8	42.2 ± 0.8**
Day 21	41.9 ± 0.2	42.0 ± 0.3	43.0 ± 0.4*	42.4 ± 0.5	44.6 ± 0.3**	45.1 ± 0.6**
Week 14	45.2 ± 0.5	44.9 ± 0.4	44.0 ± 0.9	43.3 ± 0.7	43.1 ± 0.6*	39.0 ± 1.1**
Manual hematocrit (%)						
Day 5	43.1 ± 0.4	41.9 ± 1.1	43.9 ± 0.6	43.2 ± 0.5	44.4 ± 0.7	47.4 ± 0.7**
Day 21	46.6 ± 0.3	46.5 ± 0.3	47.4 ± 0.3	46.3 ± 0.5	48.1 ± 0.3**	48.3 ± 0.7*
Week 14	48.5 ± 0.4	48.2 ± 0.5	47.1 ± 1.0	46.5 ± 0.6*	46.2 ± 0.6*	42.2 ± 1.1**
Hemoglobin (g/dL)						
Day 5	14.5 ± 0.1	14.1 ± 0.4	14.7 ± 0.2	14.6 ± 0.2	14.9 ± 0.2	15.7 ± 0.3**
Day 21	15.7 ± 0.1	15.7 ± 0.1	15.8 ± 0.1	15.4 ± 0.2	16.0 ± 0.1	16.1 ± 0.2
Week 14	15.8 ± 0.1	15.6 ± 0.1	15.2 ± 0.3*	14.9 ± 0.1**	14.6 ± 0.1**	13.6 ± 0.3**
Erythrocytes ($10^6/\mu L$)						
Day 5	6.57 ± 0.06	6.33 ± 0.22	6.64 ± 0.08	6.67 ± 0.08	6.85 ± 0.13	7.26 ± 0.14**
Day 21	7.21 ± 0.06	7.10 ± 0.05	7.27 ± 0.06	7.23 ± 0.12	8.00 ± 0.08**	8.79 ± 0.09**
Week 14	8.90 ± 0.11	8.66 ± 0.09	8.43 ± 0.17	8.45 ± 0.16	8.71 ± 0.13	8.78 ± 0.21
Reticulocytes ($10^6/\mu L$)						
Day 5	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.15 ± 0.01*	0.12 ± 0.01**	0.09 ± 0.01**
Day 21	0.13 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.08 ± 0.01*
Week 14	0.07 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
Mean cell volume (fL)						
Day 5	59.1 ± 0.2	59.9 ± 0.7	59.3 ± 0.2	58.9 ± 0.3	58.9 ± 0.3	58.1 ± 0.2*
Day 21	58.2 ± 0.3	59.2 ± 0.4	59.2 ± 0.2	58.6 ± 0.4	55.8 ± 0.3**	51.3 ± 0.3**
Week 14	50.7 ± 0.1	51.8 ± 0.3	52.3 ± 0.2	51.3 ± 0.2	49.4 ± 0.2	44.4 ± 0.4**
Mean cell hemoglobin (pg)						
Day 5	22.1 ± 0.1	22.4 ± 0.3	22.1 ± 0.2	21.9 ± 0.1	21.8 ± 0.1	21.7 ± 0.1*
Day 21	21.8 ± 0.1	22.1 ± 0.2	21.7 ± 0.1	21.4 ± 0.2	20.0 ± 0.1**	18.4 ± 0.1**
Week 14	17.7 ± 0.1	18.1 ± 0.1	18.0 ± 0.1	17.7 ± 0.2	16.8 ± 0.1**	15.5 ± 0.2**
Platelets ($10^3/\mu L$)						
Day 5	948.0 ± 41.5	979.3 ± 24.0	927.4 ± 13.5	946.4 ± 12.2	930.3 ± 21.0	890.1 ± 14.9*
Day 21	869.8 ± 41.8	886.3 ± 18.9	898.6 ± 22.5	833.4 ± 14.4*	726.6 ± 14.6**	764.7 ± 26.6**
Week 14	728.4 ± 12.3	707.0 ± 5.8	727.0 ± 25.2	716.3 ± 9.7	692.8 ± 12.6	773.4 ± 23.2

TABLE 5
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Male (continued)						
n						
Day 5	10	10	10	10	10	10
Day 21	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Clinical Chemistry						
Total protein (g/dL)						
Day 5	5.9 ± 0.1	5.8 ± 0.1	5.6 ± 0.1**	5.5 ± 0.1**	5.4 ± 0.1**	5.3 ± 0.0**
Day 21	6.7 ± 0.1	6.4 ± 0.1**	6.6 ± 0.1	6.5 ± 0.1*	6.4 ± 0.1**	6.3 ± 0.0**
Week 14	7.2 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	6.7 ± 0.1**	6.0 ± 0.1**
Albumin (g/dL)						
Day 5	4.4 ± 0.0	4.3 ± 0.0*	4.2 ± 0.0**	4.2 ± 0.1**	4.3 ± 0.0*	4.3 ± 0.0
Day 21	4.8 ± 0.0	4.7 ± 0.0	4.9 ± 0.0	5.0 ± 0.1	5.1 ± 0.0**	5.3 ± 0.0**
Week 14	5.2 ± 0.0	5.2 ± 0.1	5.3 ± 0.1	5.5 ± 0.1*	5.3 ± 0.1	5.2 ± 0.1
Cholesterol (mg/dL)						
Day 5	78 ± 1	71 ± 2*	65 ± 2**	63 ± 2**	56 ± 2**	54 ± 2**
Day 21	72 ± 2	67 ± 2	64 ± 1	57 ± 2**	63 ± 3	70 ± 4
Week 14	73 ± 2	74 ± 3	76 ± 2	67 ± 2	68 ± 2	65 ± 2*
Alanine aminotransferase (IU/L)						
Day 5	37 ± 1	39 ± 2	38 ± 1	40 ± 2	49 ± 2**	68 ± 4**
Day 21	46 ± 2	45 ± 1	46 ± 1	52 ± 2**	91 ± 4**	140 ± 10**
Week 14	48 ± 2	49 ± 2	53 ± 2	69 ± 3**	115 ± 8**	292 ± 18**
Alkaline phosphatase (IU/L)						
Day 5	639 ± 11	609 ± 18	638 ± 10	660 ± 16	759 ± 10**	781 ± 26**
Day 21	521 ± 10	515 ± 14	514 ± 10	526 ± 10	642 ± 18**	587 ± 23**
Week 14	256 ± 7	260 ± 5	248 ± 5	245 ± 6	353 ± 12**	432 ± 24**
Sorbitol dehydrogenase (IU/L)						
Day 5	17 ± 1	19 ± 1	17 ± 1	16 ± 1	18 ± 1	24 ± 2*
Day 21	13 ± 0	13 ± 1	13 ± 1	14 ± 1	28 ± 2**	40 ± 2**
Week 14	23 ± 1	27 ± 1*	26 ± 2	31 ± 1**	47 ± 2**	74 ± 4**
5′-Nucleotidase (IU/L)						
Day 5	26 ± 0	27 ± 1	27 ± 0	27 ± 1	28 ± 1	28 ± 1
Day 21	27 ± 1	28 ± 1	27 ± 0	27 ± 1	34 ± 1**	34 ± 1**
Week 14	32 ± 1	32 ± 1	29 ± 0	26 ± 1**	34 ± 1	37 ± 1
Bile acids (µmol/L)						
Day 5	36.6 ± 2.9	33.0 ± 2.9	47.7 ± 5.5	41.9 ± 6.2	72.7 ± 5.7**	151.0 ± 31.2**
Day 21	27.7 ± 4.0	29.7 ± 4.8	34.0 ± 5.8	29.3 ± 4.6	136.4 ± 32.3**	516.2 ± 59.0**
Week 14	29.2 ± 2.9	27.5 ± 2.7	27.2 ± 2.7	35.9 ± 3.9	92.0 ± 16.6**	332.4 ± 47.4**

TABLE 5
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Female						
n						
Day 5	10	10	10	10	10	10
Day 21	9	10	10	10	10	9
Week 14	10	10	10	10	10	10
Hematology						
Automated hematocrit (%)						
Day 5	42.4 ± 0.7	42.2 ± 0.5	42.2 ± 0.5	43.3 ± 0.5	44.8 ± 0.3**	46.3 ± 0.8**
Day 21	44.7 ± 0.3	45.6 ± 0.3	45.6 ± 0.5	44.0 ± 0.4	45.8 ± 0.4	44.2 ± 0.6
Week 14	42.8 ± 0.4	43.2 ± 0.4	42.1 ± 0.4	40.1 ± 0.5**	42.8 ± 0.7	34.7 ± 0.7**
Manual hematocrit (%)						
Day 5	45.6 ± 0.8	45.4 ± 0.6	44.6 ± 0.5	46.3 ± 0.6	48.1 ± 0.5*	49.9 ± 0.7**
Day 21	47.4 ± 0.3	48.1 ± 0.3	47.4 ± 0.5	46.4 ± 0.5	48.1 ± 0.5	46.0 ± 0.6
Week 14	45.8 ± 0.5	45.8 ± 0.3	44.3 ± 0.4*	42.7 ± 0.6**	45.4 ± 0.8	37.7 ± 0.6**
Hemoglobin (g/dL)						
Day 5	15.4 ± 0.2	15.5 ± 0.1	15.4 ± 0.2	15.8 ± 0.1	16.5 ± 0.1**	17.0 ± 0.2**
Day 21	16.2 ± 0.1	16.4 ± 0.1	16.3 ± 0.2	15.7 ± 0.1*	16.1 ± 0.1	15.8 ± 0.2
Week 14	15.2 ± 0.1	15.3 ± 0.1	14.9 ± 0.1	14.2 ± 0.2**	14.5 ± 0.2**	12.5 ± 0.2**
Erythrocytes ($10^6/\mu L$)						
Day 5	7.22 ± 0.12	7.17 ± 0.09	7.21 ± 0.10	7.35 ± 0.09	7.71 ± 0.05**	8.06 ± 0.14**
Day 21	7.38 ± 0.05	7.46 ± 0.05	7.60 ± 0.08*	7.51 ± 0.07*	8.34 ± 0.07**	8.54 ± 0.10**
Week 14	7.72 ± 0.08	7.71 ± 0.07	7.54 ± 0.06	7.52 ± 0.09	8.73 ± 0.16**	7.82 ± 0.13
Reticulocytes ($10^6/\mu L$)						
Day 5	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.06 ± 0.01**	0.04 ± 0.01**
Day 21	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Week 14	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Mean cell volume (fL)						
Day 5	58.7 ± 0.3	58.9 ± 0.2	58.5 ± 0.3	58.8 ± 0.2	58.0 ± 0.1*	57.5 ± 0.2**
Day 21	60.5 ± 0.4	61.2 ± 0.3	60.0 ± 0.2	58.6 ± 0.3**	55.0 ± 0.2**	51.7 ± 0.3**
Week 14	55.4 ± 0.1	56.1 ± 0.1	55.8 ± 0.1	53.3 ± 0.2*	49.0 ± 0.2**	44.4 ± 0.4**
Mean cell hemoglobin (pg)						
Day 5	21.3 ± 0.2	21.6 ± 0.1	21.4 ± 0.1	21.5 ± 0.1	21.4 ± 0.1	21.1 ± 0.2
Day 21	22.0 ± 0.1	22.1 ± 0.1	21.5 ± 0.1*	21.0 ± 0.2**	19.3 ± 0.1**	18.5 ± 0.1**
Week 14	19.7 ± 0.1	19.8 ± 0.1	19.7 ± 0.1	18.9 ± 0.1**	16.6 ± 0.2**	16.0 ± 0.2**
Platelets ($10^3/\mu L$)						
Day 5	900.3 ± 28.9	894.4 ± 23.7	842.0 ± 23.6	877.5 ± 17.1	861.9 ± 28.0	847.1 ± 29.4
Day 21	835.1 ± 17.1	813.4 ± 14.7	774.9 ± 26.1*	774.7 ± 15.6*	689.6 ± 15.1**	637.1 ± 19.8**
Week 14	742.1 ± 20.4	725.9 ± 12.7	733.9 ± 8.8	727.4 ± 14.2	639.4 ± 9.9**	662.5 ± 19.4**

TABLE 5
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Female (continued)						
n						
Day 5	10	10	10	10	10	10
Day 21	9	10	10	10	10	9
Week 14	10	10	10	10	10	10
Clinical Chemistry						
Total protein (g/dL)						
Day 5	5.8 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.6 ± 0.0	5.7 ± 0.1	5.5 ± 0.1**
Day 21	6.4 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.2 ± 0.1
Week 14	7.2 ± 0.1	7.3 ± 0.0	7.3 ± 0.1	6.9 ± 0.1	6.4 ± 0.1**	5.6 ± 0.1**
Albumin (g/dL)						
Day 5	4.4 ± 0.1	4.4 ± 0.0	4.3 ± 0.0	4.5 ± 0.0	4.5 ± 0.1	4.6 ± 0.1*
Day 21	4.8 ± 0.1	4.9 ± 0.1	5.0 ± 0.1**	5.1 ± 0.1**	5.3 ± 0.1**	5.3 ± 0.1**
Week 14	5.3 ± 0.1	5.5 ± 0.1	5.5 ± 0.0	5.4 ± 0.1	5.4 ± 0.1	4.9 ± 0.1*
Cholesterol (mg/dL)						
Day 5	108 ± 4	93 ± 1**	91 ± 3**	85 ± 2**	77 ± 2**	64 ± 2**
Day 21	96 ± 2	92 ± 1	89 ± 2**	69 ± 2**	71 ± 2**	64 ± 3**
Week 14	104 ± 4	105 ± 3	98 ± 1	81 ± 2**	64 ± 3**	55 ± 3**
Alanine aminotransferase (IU/L)						
Day 5	32 ± 1	34 ± 1	32 ± 1	41 ± 2**	54 ± 3**	69 ± 4**
Day 21	37 ± 1	37 ± 2	41 ± 2	46 ± 2**	93 ± 5**	132 ± 7**
Week 14	46 ± 2	42 ± 1	41 ± 2	49 ± 2	112 ± 7**	339 ± 18**
Alkaline phosphatase (IU/L)						
Day 5	510 ± 8	510 ± 13	492 ± 11	556 ± 14*	620 ± 16**	616 ± 18**
Day 21	432 ± 14	389 ± 15	382 ± 7	432 ± 12	545 ± 22*	453 ± 12
Week 14	227 ± 5	216 ± 4	220 ± 3	225 ± 11	341 ± 7**	468 ± 22**
Sorbitol dehydrogenase (IU/L)						
Day 5	16 ± 1	19 ± 1*	16 ± 1	16 ± 1	19 ± 2	24 ± 2**
Day 21	14 ± 1	15 ± 1	16 ± 1*	16 ± 1	34 ± 2**	41 ± 3**
Week 14	27 ± 1	27 ± 1	28 ± 2	25 ± 1	45 ± 3**	82 ± 3**
5′-Nucleotidase (IU/L)						
Day 5	33 ± 1	37 ± 1**	38 ± 1**	39 ± 1**	42 ± 1**	40 ± 2**
Day 21	35 ± 1	37 ± 1	40 ± 1	46 ± 2**	44 ± 2**	35 ± 2
Week 14	37 ± 1	38 ± 1	42 ± 1*	44 ± 2**	39 ± 1	36 ± 1
Bile acids (µmol/L)						
Day 5	24.7 ± 3.6	26.2 ± 3.5	25.4 ± 2.4	30.8 ± 3.5	51.6 ± 5.0**	102.9 ± 15.5**
Day 21	34.3 ± 3.6	21.3 ± 1.6	22.5 ± 4.0	29.3 ± 3.4	68.7 ± 10.2*	330.1 ± 52.3**
Week 14	37.0 ± 7.1	46.6 ± 6.5	39.1 ± 5.6	36.3 ± 3.9	39.3 ± 7.9	321.5 ± 50.6**

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

Exposure concentration-related hypocholesterolemia, demonstrated by decreased cholesterol concentrations, occurred at all time points in female rats. On day 5, all groups of exposed females demonstrated this effect. The number of affected groups decreased with time; at week 14, hypocholesterolemia was observed only in females exposed to 1,180 ppm or greater. On day 5, hypocholesterolemia also occurred in all groups of exposed males, but this effect waned, and there was no consistent exposure concentration relationship for decreases in cholesterol concentration in males on day 21 or at week 14. On day 5, total protein concentrations of males exposed to 589 ppm or greater and 4,600 ppm females were minimally to mildly decreased. The hypoproteinemia had ameliorated by day 21 and only occurred in 2,300 and 4,600 ppm males and females at week 14. The hypoproteinemia was accompanied by hyperalbuminemia, as evidenced by increased albumin concentrations; the increases in albumin concentrations were most pronounced on day 21 in rats exposed to 589 ppm or greater. On days 5 and 21, there was evidence of muscle injury in 2,300 and 4,600 ppm males and in females exposed to 1,180 ppm or greater, as indicated by mild increases in creatine kinase activities, especially for the 4,600 ppm groups. These differences were not apparent at week 14. Minimal decreases in the concentrations of creatinine, a marker of renal function, occurred in 2,300 ppm males and females and in 4,600 ppm females at week 14.

The thymus weights of females exposed to 4,600 ppm were significantly less than those of the vehicle controls (Table C2). The liver weights of males and females increased with increasing exposure concentration up to 1,180 ppm; at higher exposure concentrations, absolute liver weights decreased along with decreasing body weights, although relative liver weights remained increased. In males and females in the 2,300 and 4,600 ppm groups, relative kidney weights were significantly greater than those of the vehicle controls, even though absolute kidney weights were decreased in these groups.

Thin carcasses, pale livers, and liver foci were noted grossly in exposed males and females; additionally, 4,600 ppm males had small testes and seminal vesicles, and females exposed to 2,300 or 4,600 ppm had small or thin uteri. Microscopically, minimal to mild cytoplasmic vacuolization of the liver hepatocytes was observed in males and females exposed to 589, 1,180, or 2,300 ppm and males exposed to 268 ppm; this lesion did not occur in the liver of untreated or vehicle control rats (Tables 6, A1, and A2). Males and females in the 2,300 and 4,600 ppm groups also had significantly increased incidences of yellow-brown pigmentation and of hepatocyte hypertrophy and necrosis in the liver; the severity of these lesions generally increased with increasing exposure concentration. Males and females in the 4,600 ppm groups and females in the 2,300 ppm group also had significantly increased incidences of minimal to mild bile duct hyperplasia. Incidences of mild mitotic alteration of liver hepatocytes were increased in males and females exposed to 4,600 ppm; three females in the 2,300 ppm group also had this lesion. Mixed cell, basophilic, clear cell, and/or eosinophilic foci were observed in the liver of males and females in the 2,300 ppm and 4,600 ppm groups. The incidences of mixed cell foci in males in the 4,600 ppm group and females in the 2,300 ppm group and of eosinophilic foci in females in the 2,300 ppm group were significantly increased.

TABLE 6
Incidences of Selected Nonneoplastic Lesions in Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Male						
Liver[a]	10	10	10	10	10	10
Hepatocyte, Vacuolization Cytoplasmic[b]	0	7** (1.3)[c]	9** (2.0)	10** (1.9)	8** (1.4)	0
Hepatocyte, Hypertrophy	0	0	0	1 (1.0)	9** (1.3)	10** (3.2)
Hepatocyte, Necrosis	0	0	0	0	8** (1.0)	10** (1.6)
Pigmentation	0	0	0	0	7** (1.0)	10** (1.9)
Bile Duct, Hyperplasia	0	0	0	0	0	10** (1.7)
Hepatocyte, Mitotic Alteration	0	0	0	0	0	6** (2.0)
Mixed Cell Focus	0	0	0	0	3	5*
Eosinophilic Focus	0	0	0	0	1	1
Clear Cell Focus	0	0	0	0	1	1
Basophilic Focus	0	0	0	0	0	3
Spleen	10	10	10	10	10	10
Pigmentation	0	0	1 (1.0)	9** (1.0)	9** (1.0)	9** (1.6)
Red Pulp, Atrophy	0	0	0	0	5* (1.0)	9** (1.4)
Lymphoid Follicle, Atrophy	0	0	0	0	0	5* (1.0)
Bone	10	10	9	10	10	10
Metaphysis, Atrophy	0	0	0	0	0	10** (2.1)
Bone Marrow	10	10	9	10	10	10
Atrophy	0	0	0	0	3 (1.0)	10** (1.5)
Prostate Gland	10	10	9	10	10	10
Atrophy	0	0	0	0	0	9** (2.0)
Preputial Gland	10	5	5	6	10	10
Atrophy	0	0	0	0	0	10** (1.4)
Seminal Vesicle	10	10	9	10	10	10
Atrophy	0	0	0	0	0	10** (2.7)
Testes	10	9	9	10	10	10
Germinal Epithelium, Atrophy	0	0	2 (1.5)	0	0	10** (2.2)

TABLE 6
Incidences of Selected Nonneoplastic Lesions in Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Female						
Liver	10	10	10	10	10	10
Hepatocyte, Vacuolization Cytoplasmic	0	0	10** (1.7)	10** (2.2)	4* (1.3)	0
Hepatocyte, Hypertrophy	0	0	0	4* (1.0)	10** (1.7)	10** (2.8)
Hepatocyte, Necrosis	0	0	0	1 (1.0)	7** (1.0)	10** (1.1)
Pigmentation	0	0	0	0	10** (1.3)	10** (2.0)
Bile Duct, Hyperplasia	0	0	0	0	5* (1.0)	10** (1.9)
Hepatocyte, Mitotic Alteration	0	0	0	0	3 (2.0)	10** (1.9)
Mixed Cell Focus	0	0	0	0	8**	1
Eosinophilic Focus	0	0	0	0	4*	2
Clear Cell Focus	0	0	0	0	2	3
Basophilic Focus	0	0	0	0	1	0
Spleen	10	10	10	10	10	10
Pigmentation	1 (1.0)	0	0	4 (1.0)	8** (1.1)	8** (1.3)
Red Pulp, Atrophy	0	0	0	0	0	9** (1.6)
Lymphoid, Follicle, Atrophy	0	0	0	0	0	3 (1.0)
Bone	10	10	10	10	10	10
Metaphysis, Atrophy	0	0	0	0	9** (1.8)	9** (2.9)
Bone Marrow	10	10	10	10	10	10
Atrophy	0	0	0	0	4* (1.0)	7** (1.7)
Uterus	10	10	10	10	10	10
Atrophy	0	0	0	0	7** (1.4)	9** (2.2)
Ovary	10	10	10	10	10	10
Interstitial Cell, Cytoplasmic Alteration	0	0	0	0	3 (1.0)	10** (2.0)
Clitoral Gland	9	7	7	10	10	10
Atrophy	0	0	0	0	1 (1.0)	7** (1.4)

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

Males and females exposed to 1,180 ppm or greater had significantly increased incidences of minimal to mild hemosiderin pigmentation in the spleen (Tables 6, A1, and A2). Males in the 2,300 ppm group and males and females in the 4,600 ppm groups had significantly increased incidences of minimal to mild atrophy of the splenic red pulp. Minimal atrophy of the lymphoid follicle of the spleen was observed in males and females exposed to 4,600 ppm, and

the incidence in males was significantly increased. Significantly increased incidences of atrophy of the bone metaphysis and bone marrow occurred in males and females in the 4,600 ppm groups and females in the 2,300 ppm group; three males in the 2,300 ppm group also had bone marrow atrophy. The average severity of atrophy was minimal to mild in males and minimal to moderate in females.

Males exposed to 4,600 ppm had minimal to moderate atrophy of the prostate gland, preputial gland, seminal vesicle, and testicular germinal epithelium (Tables 6 and A1). Minimal to mild atrophy of the uterus and clitoral gland and cytoplasmic alteration of the ovarian interstitial cell were observed in females in the 2,300 and 4,600 ppm groups, and the incidences of these lesions in the 4,600 ppm group and of uterine atrophy in the 2,300 ppm group were significantly greater than those in the vehicle controls (Tables 6 and A2).

Epididymal spermatozoal motility values of males exposed to 589 ppm or greater, the left cauda epididymis and left epididymis weights of males in the 2,300 ppm group, and the left epididymis weight of males in the 1,180 ppm group were significantly less than those of the vehicle controls (Table D1). Females in the 2,300 ppm group spent more time in diestrus and less time in proestrus, estrus, and metestrus than did the vehicle control females (Table D2).

MICE

15-DAY STUDY

All male and female mice exposed to 53,200 ppm, all males exposed to 26,600 ppm, and two males exposed to 13,300 ppm died or were killed moribund before the end of the study (Table 7). The final mean body weights and body weight gains of all exposed groups of males and females with survivors were significantly less than those of the vehicle controls; males in all exposed groups with survivors and females exposed to 6,650 ppm or greater lost weight during the study (Table 7). Due to excessive scattering of feed, especially by the higher exposed groups, feed consumption could not be measured accurately. Therefore, no average daily doses have been calculated. Clinical findings included hyperactivity in males and females in the 3,325, 6,650, and 13,300 ppm groups and females in the 26,600 ppm group; males in the 26,600 and 53,200 ppm groups were lethargic. Males exposed to 6,650 ppm or greater and females exposed to 26,600 or 53,200 ppm were thin and had ruffled fur.

Absolute and relative thymus weights of all exposed groups of females were significantly less than those of the vehicle controls (Table C3). Relative liver weights of exposed groups of male mice were significantly greater than that of the vehicle control group. Other changes in organ weights generally reflected body weight changes.

At necropsy, thin carcasses were noted in males exposed to 6,650 or 13,300 ppm and females exposed to 13,300 or 26,600 ppm. Pale or mottled livers were noted in all groups of exposed males and females and correlated microscopically with hepatocellular degeneration characterized by hepatocellular swelling, cytoplasmic rarefaction, single paranuclear vacuoles, hepatocellular necrosis with occasional pooling of sinusoidal erythrocytes, and infrequent mild mononuclear infiltrates. The severity of hepatocellular degeneration and hepatocellular swelling with cytoplasmic rarefaction increased with increasing exposure concentration.

Based on the early deaths of mice exposed to 26,600 or 53,200 ppm and the decreased body weight gains of mice exposed to 3,325 ppm or greater, the exposure concentrations selected for mice in the 14-week study were 589, 1,120, 2,300, 4,550, and 9,100 ppm.

Table 7
Survival, Body Weights, and Feed Consumption of Mice in the 15-Day Feed Study of 1,1,2,2-Tetrachloroethane

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Vehicle Controls (%)	Feed Consumption[c]
Male						
Untreated Control	5/5	23.0 ± 0.4	25.0 ± 0.5	2.0 ± 0.4		4.4
Vehicle Control	5/5	23.1 ± 0.4	24.4 ± 0.3	1.3 ± 0.2		4.4
3,325	5/5	22.4 ± 0.8	20.9 ± 0.7**	−1.5 ± 0.8*	86	3.9
6,650	5/5	22.7 ± 0.7	19.0 ± 0.6**	−3.7 ± 1.2**	78	4.7
13,300	3/5[d]	22.8 ± 0.6	19.6 ± 0.6**	−2.9 ± 0.2**	80	8.6
26,600	0/5[e]	22.9 ± 0.8	—	—	—	—
53,200	0/5[f]	22.9 ± 0.8	—	—	—	—
Female						
Untreated Control	5/5	18.1 ± 0.5	21.1 ± 0.5	3.0 ± 0.1		3.3
Vehicle Control	5/5	18.0 ± 0.5	21.3 ± 0.4	3.3 ± 0.3		2.9
3,325	5/5	17.6 ± 0.6	19.2 ± 0.5**	1.6 ± 0.3**	90	3.3
6,650	5/5	17.6 ± 0.4	16.8 ± 0.2**	−0.8 ± 0.3**	79	4.3
13,300	5/5	17.7 ± 0.5	14.6 ± 0.3**	−3.1 ± 0.5**	69	4.2
26,600	5/5	18.0 ± 0.5	13.8 ± 0.3**	−4.1 ± 0.4**	65	4.2
53,200	0/5[g]	15.7 ± 0.4*	—	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Number of animals surviving at 15 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. No final mean body weights, weight changes, or feed consumption were calculated for groups with 100% mortality.
[c] Feed consumption values (expressed as grams of feed consumed per animal per day) are inaccurate due to excessive scattering of feed.
[d] Day of death: 6, 6
[e] Day of death: 5, 5, 5, 6, 6
[f] Day of death: 4, 4, 4, 5, 5
[g] Day of death: 6, 7, 7, 7, 7

14-WEEK STUDY

All mice survived to the end of the studies (Table 8). The final mean body weights and body weight gains of males and females exposed to 2,300 ppm or greater were generally less than those of the vehicle controls; females in the 589 and 1,120 ppm groups also had significantly lower mean body weight gains than the untreated controls (Table 8 and Figure 2). Feed consumption by males exposed to 4,550 or 9,100 ppm was slightly less than that by the untreated and vehicle controls; feed consumption by exposed females was similar to that by the untreated and vehicle controls. Exposure concentrations of 589, 1,120, 2,300, 4,550, and 9,100 ppm resulted in average daily doses of 100, 200, 370, 700, and 1,360 mg/kg for males and 80, 160, 300, 600, and 1,400 mg/kg for females. Clinical findings included thinness of three males and one female exposed to 2,300 ppm, nine males and two females exposed to 4,550 ppm, and all males and females exposed to 9,100 ppm. Results of the functional observation battery indicated no exposure-related findings of neurotoxicity.

TABLE 8
Survival, Body Weights, and Feed Consumption of Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Vehicle Controls (%)	Feed Consumption[c] Week 2	Week 13
Male							
Untreated Control	10/10	20.8 ± 0.5	30.4 ± 0.8	9.6 ± 0.5			
Vehicle Control	10/10	20.4 ± 0.4	30.1 ± 0.6	9.7 ± 0.4			
589	10/10	20.6 ± 0.3	30.6 ± 0.6	10.1 ± 0.5	102	3.8	4.3
1,120	10/10	20.5 ± 0.4	30.0 ± 0.3	9.5 ± 0.3	99	4.0	4.7
2,300	10/10	20.8 ± 0.6	26.5 ± 0.4**	5.7 ± 0.5**	88	3.5	4.1
4,550	10/10	20.6 ± 0.4	25.2 ± 0.2**	4.6 ± 0.4**	84	3.1	4.0
9,100	10/10	20.5 ± 0.4	23.1 ± 0.5**	2.6 ± 0.5**	77	2.9	3.6
Female							
Untreated Control	10/10	16.8 ± 0.3	25.6 ± 0.8	8.8 ± 0.6			
Vehicle Control	10/10	16.8 ± 0.4	24.3 ± 0.5	7.4 ± 0.4			
589	10/10	16.7 ± 0.4	24.2 ± 0.2	7.5 ± 0.3	100	2.5	3.0
1,120	10/10	16.6 ± 0.4	24.3 ± 0.6	7.8 ± 0.4	100	2.7	3.2
2,300	10/10	16.8 ± 0.4	23.3 ± 0.4	6.4 ± 0.3*	96	2.5	2.9
4,550	10/10	16.4 ± 0.3	21.7 ± 0.2**	5.3 ± 0.3**	90	2.3	2.9
9,100	10/10	17.0 ± 0.4	21.5 ± 0.6**	4.5 ± 0.4**	89	3.0	3.0

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.

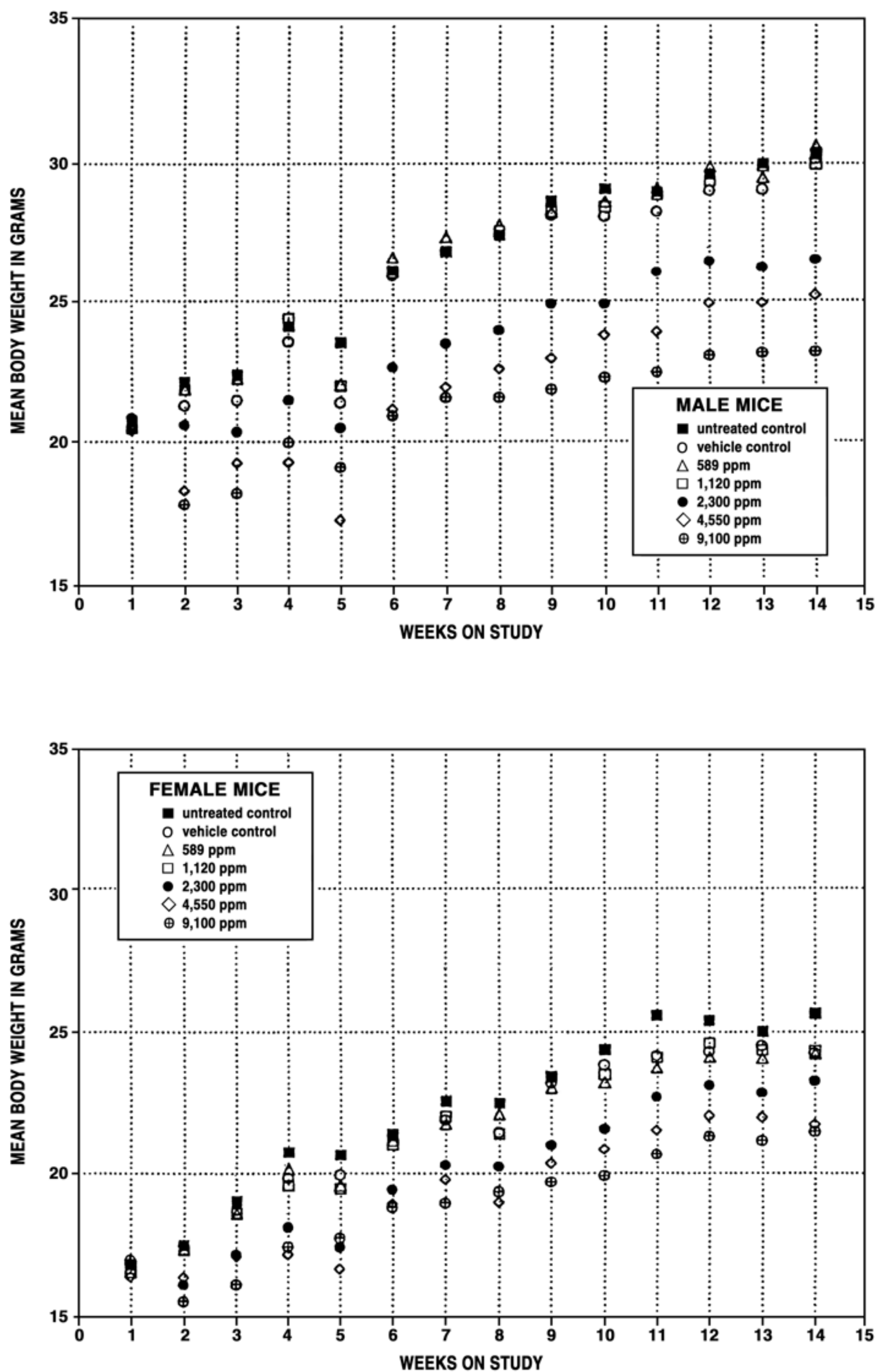


Figure 2
Body Weights of Male and Female Mice Exposed to 1,1,2,2-Tetrachloroethane in Feed for 14 Weeks

Clinical chemistry data for mice are listed in Tables 9 and B2. As in the rat study at week 14, an exposure concentration-related hepatic effect in mice was indicated by increases in alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, and 5′-nucleotidase activities and total bile acid concentrations in males and females exposed to 1,120 ppm or greater. Also similar to the rat study, there were treatment-related decreases in total protein and cholesterol concentrations in mice. Decreased total protein concentrations occurred in males and females exposed to 2,300 ppm or greater and in 1,120 ppm males; cholesterol concentrations were decreased in males in the 1,120 ppm group and in females exposed to 1,120 ppm or greater. Except for the alterations in total protein concentrations in female mice, the decreased total protein and cholesterol concentrations did not demonstrate exposure concentration relationships. In 9,100 ppm males, the minimally decreased protein concentration was accompanied by a minimally increased albumin concentration, which suggested that males in this group may have been slightly dehydrated, masking the severity of hypoproteinemia. In 9,100 ppm females, the decrease in total protein concentration was accompanied by a concomitant decrease in albumin concentration.

The liver weights of males in the 1,120 and 2,300 ppm groups and females in all exposed groups were generally significantly greater than those of the untreated and vehicle controls (Table C4). Kidney weights of males exposed to 2,300 ppm or greater were significantly less than those of the vehicle controls. The absolute thymus weights of males and females in the 9,100 ppm group were less than those of the vehicle controls.

At necropsy, thin carcasses were noted for males and females in the 4,550 and 9,100 ppm groups. Males exposed to 2,300 ppm or greater and all exposed groups of females had pale livers; pale kidneys were also observed in one male in each of the 4,550 and 9,100 ppm groups.

Microscopically, males and females exposed to 1,120 ppm or greater had significantly greater incidences of minimal to moderate hypertrophy of the liver hepatocytes than did the vehicle controls; two females in the 589 ppm group also had this lesion (Tables 10, A3, and A4). Incidences of hepatocyte necrosis, focal pigmentation, and bile duct hyperplasia were generally significantly greater in males and females exposed to 2,300 ppm or greater than in the untreated or vehicle controls. Preputial gland atrophy was significantly increased in males in the 589, 4,550, and 9,100 ppm groups compared to the untreated and vehicle controls (Tables 10 and A3).

The left cauda epididymis, left epididymis, and left testis weights of males in the 9,100 ppm group and the left testis weight of males in the 4,550 ppm group were significantly less than those of the vehicle controls (Table D3). The epididymal spermatozoal motility of males in the 9,100 ppm group was significantly less than that of the vehicle controls. The estrous cycle of females in the 9,100 ppm group was longer than that of the vehicle controls (Table D4).

TABLE 9
Selected Clinical Chemistry Data for Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Vehicle Control	589 ppm	1,120 ppm	2,300 ppm	4,550 ppm	9,100 ppm
n	10	10	10	10	10	10
Male						
Total protein (g/dL)	5.4 ± 0.1	5.2 ± 0.1	5.1 ± 0.1**	5.1 ± 0.1**	5.1 ± 0.1*	5.1 ± 0.1**
Albumin (g/dL)	3.8 ± 0.0	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.0	3.8 ± 0.0	3.9 ± 0.1
Cholesterol (mg/dL)	131 ± 7	125 ± 4	94 ± 3**	110 ± 5	112 ± 4	126 ± 5
Alanine aminotransferase (IU/L)	66 ± 8	62 ± 19	74 ± 8	207 ± 18**	172 ± 18**	296 ± 24**
Alkaline phosphatase (IU/L)	85 ± 2	78 ± 2	89 ± 2	130 ± 3**	143 ± 7**	184 ± 11**
Sorbitol dehydrogenase (IU/L)	55 ± 3	53 ± 2	76 ± 3**	288 ± 20**	288 ± 29**	448 ± 25**
5′-Nucleotidase (IU/L)	18 ± 1	16 ± 1	18 ± 0	30 ± 2**	37 ± 3**	62 ± 7**
Bile acids (µmol/L)	25.3 ± 1.2	22.8 ± 1.5	24.8 ± 0.6	56.5 ± 5.1**	63.3 ± 7.5**	108.7 ± 8.1**
Female						
Total protein (g/dL)	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.0	5.4 ± 0.1*	5.4 ± 0.0**	5.1 ± 0.1**
Albumin (g/dL)	4.3 ± 0.1	4.5 ± 0.1	4.4 ± 0.0	4.3 ± 0.1	4.3 ± 0.0	4.0 ± 0.0**
Cholesterol (mg/dL)	109 ± 2	109 ± 3	85 ± 3**	68 ± 2**	64 ± 3**	92 ± 4**
Alanine aminotransferase (IU/L)	34 ± 5	50 ± 15	65 ± 5**	189 ± 33**	197 ± 21**	351 ± 35**
Alkaline phosphatase (IU/L)	131 ± 5	126 ± 2	139 ± 5	150 ± 3**	161 ± 7**	195 ± 6**
Sorbitol dehydrogenase (IU/L)	36 ± 1	44 ± 3*	76 ± 4**	197 ± 15**	243 ± 23**	461 ± 59**
5′-Nucleotidase (IU/L)	59 ± 3	71 ± 2	84 ± 5**	62 ± 2	62 ± 3	83 ± 4**
Bile acids (µmol/L)	27.2 ± 1.2	26.1 ± 1.9	30.9 ± 1.1*	44.2 ± 3.9**	51.5 ± 3.6**	101.7 ± 12.0**

* 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.

TABLE 10
Incidences of Selected Nonneoplastic Lesions in Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Vehicle Control	589 ppm	1,120 ppm	2,300 ppm	4,550 ppm	9,100 ppm
Male						
Liver[a]	10	10	10	10	10	10
Hepatocyte, Hypertrophy[b]	0	0	7** (1.0)[c]	10** (2.2)	10** (2.8)	10** (3.1)
Hepatocyte, Necrosis	0	0	1 (2.0)	8** (1.1)	8** (1.0)	9** (1.0)
Pigmentation, Focal	0	0	0	10** (1.2)	10** (1.4)	8** (1.3)
Bile Duct, Hyperplasia	0	0	0	7** (1.4)	9** (1.3)	10** (2.0)
Preputial Gland	10	10	10	8	10	9
Atrophy	0	4* (2.0)	2 (1.0)	0	4* (2.5)	5* (2.2)
Female						
Liver	10	10	10	10	10	10
Hepatocyte, Hypertrophy	0	2 (1.5)	9** (1.0)	10** (1.9)	10** (2.5)	10** (3.0)
Hepatocyte, Necrosis	0	0	0	3 (1.0)	7** (1.0)	4* (1.0)
Pigmentation, Focal	0	0	2 (1.0)	9** (1.0)	8** (1.0)	7** (1.1)
Bile Duct, Hyperplasia	0	0	0	8** (1.0)	10** (1.4)	10** (2.0)

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

GENETIC TOXICOLOGY

The evidence for genotoxic activity of 1,1,2,2-tetrachloroethane is mixed, with consistently negative results reported in gene mutation assays but with positive and negative results observed in chromosomal damage assays. Combined results from two independent studies showed that 1,1,2,2-tetrachloroethane was negative for induction of mutations in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 with and without induced rat, hamster, and mouse liver S9 activation enzymes (Table E1; Haworth *et al.*, 1983). Negative results were also obtained with 1,1,2,2-tetrachloroethane in the L5178Y mouse lymphoma cell gene mutation assay with and without S9 (Table E2). Additional testing in mammalian cells demonstrated induction of sister chromatid exchanges (Table E3), but not chromosomal aberrations (Table E4), in Chinese hamster ovary cells cultured with 1,1,2,2-tetrachloroethane in the presence and the absence of S9 (Galloway *et al.*, 1987); similar concentrations of 1,1,2,2-tetrachloroethane were used in these *in vitro* cytogenetic tests.

In vivo, negative results were obtained for induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* after administration of 1,1,2,2-tetrachloroethane to adult flies via feeding or injection (Table E5; Woodruff *et al.*, 1985). Positive results were obtained in a peripheral blood micronucleus assay conducted in male and female mice at the end of the 14-week feed study (Table E6). Trend analyses on the frequencies of micronucleated normochromatic erythrocytes in males and females were positive ($P \leq 0.001$ for males and $P=0.008$ for females), but only the two highest concentrations tested for males yielded micronucleated normochromatic erythrocyte frequencies that were significantly different from the vehicle control values by pairwise comparisons.

In conclusion, negative results were obtained with 1,1,2,2-tetrachloroethane in gene mutation assays in *S. typhimurium*, cultured mouse lymphoma cells, and germ cells of male *D. melanogaster*. Tests for induction of chromosomal effects in mammalian cells yielded mixed results, with positive results in a sister chromatid exchange test and negative results in a chromosomal aberrations test *in vitro* and positive results in an *in vivo* peripheral blood micronucleus test in male and female mice.

DISCUSSION

Although 1,1,2,2-tetrachloroethane is known to be carcinogenic when administered to B6C3F_1 mice by gavage at a dose of 142 or 282 mg/kg body weight per day for 78 weeks (NCI, 1978), the present studies were conducted by administering microencapsulated 1,1,2,2-tetrachloroethane in feed. The purpose of the dosed feed studies was to determine if toxic effects were manifested when the chemical was not administered in bolus doses, because the general public is likely to be exposed to the chemical in contaminated drinking water. The F344/N rat studies were included because the NCI (1978) studies in Osborne-Mendel rats were considered inconclusive.

In the 15-day feed study in rats, body weight gains were reduced in all groups of exposed males and females. In the 14-week study, body weight gains were significantly reduced in rats exposed to concentrations of 1,180 ppm or greater compared to the vehicle controls. The body weight effects were probably related to reduced feed consumption.

The hematology results of the 14-week feed study in rats indicated that exposure of rats to 1,1,2,2-tetrachloroethane affected the circulating erythroid mass. There was evidence of a transient erythrocytosis in the exposed rats, suggesting a physiological response consistent with the hemoconcentration of dehydration. Because there were marked reductions in body weight gains in rats exposed to 1,180 ppm or greater, it could be hypothesized that the treated animals were not eating properly and, thus, not drinking properly. The minimal to mild increases in albumin concentrations which occurred in animals in the exposed groups at various time points would also be consistent with dehydration (Kaneko, 1989). Decreased reticulocyte counts may have been related to the erythrocytosis. Because erythropoiesis in rats is sensitive to protein intake (Bethard *et al.*, 1958), the decreased feed consumption may also have contributed to the decreased reticulocyte counts. The minimal severity of the erythrocytosis was not considered clinically significant but may have masked the severity of the anemia that occurred at week 14 and, possibly, an anemia at the interim time points.

At the end of the study, the erythrocytosis was replaced by evidence of a minimal to mild exposure concentration-related anemia. The lack of an erythroid response to the anemia was supported by the bone marrow atrophy observed microscopically. Suppression anemias have been associated with prolonged deficiencies of protein and certain vitamins and minerals essential for erythropoiesis and, secondarily, to chemical toxicity and prolonged inflammatory or organic disease (Jain, 1986). Because there were reductions in weight gains and feed consumption in rats in the higher exposure groups, it could be hypothesized that the nutritional status of the treated animals was compromised and may have, at least in part, contributed to the development of anemia.

Based on the minimal to mild, exposure concentration-related decreases in mean cell volumes and mean cell hemoglobin values, the anemia was also characterized as microcytic. Microcytic anemia has occurred in cases of iron, copper, or pyridoxine deficiencies; administration of gallium compounds; and lead toxicosis (Jain, 1986; Smith, 1989; Gurer *et al.*, 1998; NTP, 2000). Therefore, in this study, microcytosis would suggest an alteration in iron metabolism or hemoglobin production.

Treatment-related decreases in platelet counts occurred in different exposed groups of males and females on days 5 and 21 and at week 14. Bone marrow atrophy would be consistent with decreased platelet production. The decreased platelet counts indicated a biological effect but were of a mild severity that would not have been expected to lead to a clinical hemorrhagic diathesis.

Relative liver weights were significantly increased in males and females exposed to 589 ppm or greater. The enlarged livers in the lower exposure groups were accompanied by cytoplasmic vacuolization of hepatocytes. In males and females in the 2,300 and 4,600 ppm groups, hepatocyte hypertrophy and necrosis were observed. In 4,600 ppm males and in 2,300 and 4,600 ppm females, bile duct hyperplasia and mitotic alteration of hepatocytes were also observed. Treatment-related increases in alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, and 5′-nucleotidase activities and bile acid concentrations would indicate a hepatic effect and were consistent with the histopathologic liver alterations and increases in liver weights. In general, increases in serum activities of alanine aminotransferase and sorbitol dehydrogenase, considered to be liver-specific enzymes in rodents, are used as markers of hepatocellular necrosis or increased cell membrane permeability (Clampitt and Hart, 1978; Boyd, 1983). Increases in alkaline phosphatase and 5′-nucleotidase activities and bile acid concentrations are used as markers of cholestasis (Issa *et al.*, 1976; Hoffmann *et al.*, 1989). Altered enterohepatic circulation and impaired hepatocellular function or hepatocellular injury can elevate circulating bile acid concentrations (Hofmann, 1988).

In the NCI (1978) rat studies, nonneoplastic lesions in the liver were unremarkable; the combined incidence of neoplastic liver nodule and hepatocellular carcinoma (3/49 or 6%) in male rats was considered inconclusive. The histopathologic changes observed in the rat liver in the present study would not preclude a hepatocarcinogenic effect of 1,1,2,2-tetrachloroethane in rats in 2-year studies.

In the current 14-week studies, no histopathologic changes were observed in the kidney, nor were they observed in the kidney in the NCI (1978) studies. Atrophy of the bone, bone marrow, and reproductive organs was observed in 4,600 ppm males and females and in 2,300 ppm females. The atrophy may, in part, be related to the reduction in body weight gains during the course of the 14-week treatment.

In the 15-day feed study in mice, all groups of exposed mice lost weight except 3,325 ppm females. All males and females administered 53,200 ppm died within 4 to 7 days; all males exposed to 26,600 ppm died in 5 or 6 days. All 26,600 ppm females survived in spite of body weight loss. Relative liver weights in exposed males and females were generally significantly increased, even though absolute liver weights in females were less than those of the vehicle controls. The enlarged livers were accompanied by hepatocellular degenerative changes.

In the NCI (1978) gavage studies, 142 or 282 mg/kg 1,1,2,2-tetrachloroethane was administered orally to mice for 78 weeks. In a study by Paolini *et al.* (1992), a single dose (300 or 600 mg/kg) of the chemical in corn oil was administered intraperitoneally to male and female Swiss (CD-1®) albino mice. Therefore, even though the body weight gains of all exposed groups of mice in the current 15-day study were significantly less than those of the vehicle controls, it was felt that mice could tolerate a greater dose in feed, and the highest exposure concentration selected for the 14-week study was 9,100 ppm.

All mice in the 14-week study survived, but body weight gains of males and females exposed to 2,300 ppm or greater were significantly less than those of the vehicle controls. Liver weights of males in the 1,120 and 2,300 ppm groups and females in all exposed groups were significantly greater than those in the vehicle controls. Histologic examination revealed hepatocyte hypertrophy in the liver of males and females exposed to 1,120 ppm or greater; this lesion was accompanied by necrosis, focal pigmentation, and bile duct hyperplasia. In the NCI (1978) gavage studies, male and female B6C3F$_1$ mice developed high incidences of hepatocellular carcinoma.

The NCI (1978) reported that gavage administration of 142 or 282 mg/kg 1,1,2,2-tetrachloroethane for 78 weeks induced hepatocellular carcinoma in B6C3F$_1$ mice but not in Osborne-Mendel rats. Mitoma *et al.* (1985) examined the metabolism, hepatic protein binding, and urinary metabolite patterns of the chemical in Osborne-Mendel rats and B6C3F$_1$ mice and reported that metabolism and hepatic protein binding were greater in mice than in rats. There were no differences in the urinary metabolite pattern of the chemical between rats and mice. Mitoma *et al.* (1985) speculated that the higher metabolic rate and protein binding of 1,1,2,2-tetrachloroethane in mice than in rats probably accounted for the species difference in the carcinogenic response. However, Colacci *et al.* (1987) showed that DNA binding of 1,1,2,2-tetrachloroethane to mouse liver DNA was greater than that in rat liver DNA. In the present 15-day feed studies, male and female rats and male mice exposed to 26,600 ppm died, but female mice survived. In the 14-week studies, body weight gains of 1,180 ppm male and female rats were less than those of the vehicle controls, whereas 1,120 ppm 1,1,2,2-tetrachloroethane had no effect on body weights of male or female mice. The incidences of hepatic hypertrophy were greater in 1,120 ppm mice than in 1,180 ppm rats. The data seem to indicate that mice could tolerate 1,1,2,2-tetrachloroethane better than rats, but their livers were more sensitive to the effect of 1,1,2,2-tetrachloroethane.

Serum concentrations of cholesterol were decreased in exposed male and female rats and female mice. These decreases in cholesterol concentrations were exposure concentration related in rats. The liver was identified as a target tissue in these studies, however, and it is the major site of cholesterol biosynthesis in the rat (Bartley, 1989). Therefore, factors affecting the activity of HMG-CoA reductase (the rate-limiting enzyme of cholesterol synthesis) in the liver, such as decreased HMG-CoA reductase production, production of biologically inactive enzyme, increased degradation, or inhibition, would affect circulating concentrations of cholesterol. Additionally, because high-density lipoprotein (HDL) carries about 60% of the circulating cholesterol in the rat (Carroll and Feldman, 1989), a decrease in HDL concentrations could affect serum total cholesterol concentrations. Finally, the nutritional status of the animals could have affected cholesterol metabolism. Dietary manipulations (Carroll and Feldman, 1989) and dietary restriction (Imai *et al.*, 1991) can affect circulating cholesterol concentrations in rats. The nutritional status of the exposed animals may have, at least in part, contributed to the hypocholesterolemia.

There was evidence of a minimal to moderate hypoproteinemia in the rat and mouse studies. Often, decreases in serum total protein concentrations are related to decreases in serum albumin concentrations. In these studies, however, decreases in total protein concentrations occurred more frequently than were explicable by decreases in albumin concentration alone; in fact, in many instances albumin concentrations were increased (hyperalbuminemia). These findings would suggest that other protein fractions, such as globulins derived from the liver or immunoglobulins from lymphocytes, were affected. Hyperalbuminemia has not been associated with increased production but has been used as an indicator of dehydration (Kaneko, 1989). The hydration status of the exposed animals may have been affected due to decreased water consumption, resulting in dehydration and subsequent hyperalbuminemia and, possibly, masking an actual hypoalbuminemia. Dehydration could account for the amelioration of the hypoproteinemia on day 21 and may have masked the severity of the hypoproteinemia and the anemia. Since there was biochemical and morphological evidence of hepatic injury, altered hepatic function may have contributed to the hypoproteinemia. Additionally, because nutrition plays an important role in protein status, treatment-related decreases in feed intake could also account for some of the low protein values.

Creatine kinase is considered a tissue-specific indicator of cardiac and skeletal muscle injury and has been used for the detection of myopathies in many species (Cardinet, 1989; Hoffmann *et al.*, 1989; Kramer, 1989). Increases in creatine kinase activity can occur for physiological and pathological reasons. The etiology for the increased creatine kinase activities in the present studies is unknown, but because creatine kinase has a short biological half-life, the transient increases would suggest that the insult was not persistent. Additionally, there were no morphologic lesions observed in cardiac or skeletal muscle in the rats; therefore, the mild, transient increases in creatine kinase activities observed in these studies would suggest that the injury was not clinically important.

It has been demonstrated that serum creatinine concentrations are related to muscle mass (Finco, 1989; Ragan, 1989). In the 14-week study, rats exposed to 1,180 ppm weighed less than did vehicle control rats; thus, the decreases in creatinine concentration in 2,300 ppm males and females and in 4,600 ppm females would be consistent with muscle mass differences between the vehicle control and exposed animals.

Based on the survival and body weight effects and increased lesion incidences observed in the present studies, the no-observed-adverse-effect level (NOAEL) for 1,1,2,2-tetrachloroethane was estimated to be 268 ppm for rats. Because the NCI (1978) reported that the chemical is carcinogenic in mice, no NOAEL was estimated for mice in the present studies.

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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 14-Week Feed Study of 1,1,2,2-Tetrachloroethane A-2

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane A-5

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane A-8

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane A-10

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Survivors							
Died last week of study							3
Terminal sacrifice	10	10	10	10	10	10	7
Animals examined microscopically	10	10	10	10	10	10	10
Alimentary System							
Intestine large, rectum	(10)	(10)					(10)
Artery, thrombosis		1 (10%)					
Liver	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Basophilic focus							2 (20%)
Basophilic focus, multiple							1 (10%)
Clear cell focus						1 (10%)	1 (10%)
Eosinophilic focus						1 (10%)	1 (10%)
Hematopoietic cell proliferation				1 (10%)			
Hepatodiaphragmatic nodule		1 (10%)		2 (20%)		2 (20%)	1 (10%)
Mixed cell focus						3 (30%)	5 (50%)
Pigmentation						7 (70%)	10 (100%)
Bile duct, hyperplasia							10 (100%)
Hepatocyte, hypertrophy					1 (10%)	9 (90%)	10 (100%)
Hepatocyte, mitotic alteration							6 (60%)
Hepatocyte, necrosis						8 (80%)	10 (100%)
Hepatocyte, vacuolization cytoplasmic			7 (70%)	9 (90%)	10 (100%)	8 (80%)	
Pancreas	(10)	(10)					(10)
Acinus, atrophy, focal	1 (10%)	3 (30%)					1 (10%)
Stomach, forestomach	(10)	(10)					(10)
Inflammation, acute							1 (10%)
Cardiovascular System							
Heart	(9)	(10)					(10)
Cardiomyopathy	4 (44%)	9 (90%)					2 (20%)
Endocrine System							
Adrenal cortex	(10)	(10)					(10)
Inflammation, chronic, focal		1 (10%)					
Pituitary gland	(10)	(10)					(10)
Pars distalis, cyst	1 (10%)						
Thyroid gland	(10)	(10)					(10)
Ultimobranchial cyst		1 (10%)					
General Body System							
None							

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

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Genital System							
Preputial gland	(10)	(10)	(5)	(5)	(6)	(10)	(10)
Atrophy							10 (100%)
Inflammation, chronic	4 (40%)	5 (50%)				3 (30%)	3 (30%)
Inflammation, chronic active	1 (10%)		1 (20%)			1 (10%)	
Bilateral, inflammation, chronic							2 (20%)
Prostate	(10)	(10)	(10)	(9)	(10)	(10)	(10)
Atrophy							9 (90%)
Inflammation, acute		1 (10%)				1 (10%)	
Inflammation, chronic active		1 (10%)					
Seminal vesicle	(10)	(10)	(10)	(9)	(10)	(10)	(10)
Atrophy							10 (100%)
Testes	(10)	(10)	(9)	(9)	(10)	(10)	(10)
Germinal epithelium, atrophy				2 (22%)			10 (100%)
Hematopoietic System							
Bone marrow	(10)	(10)	(10)	(9)	(10)	(10)	(10)
Atrophy						3 (30%)	10 (100%)
Lymph node, mandibular	(10)	(10)					(10)
Hemorrhage	2 (20%)						
Hyperplasia, lymphoid	2 (20%)						
Lymph node, mesenteric	(9)	(10)					(10)
Infiltration cellular, histiocyte		2 (20%)					
Spleen	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Pigmentation				1 (10%)	9 (90%)	9 (90%)	9 (90%)
Lymphoid follicle, atrophy							5 (50%)
Red pulp, atrophy						5 (50%)	9 (90%)
Thymus	(10)	(10)		(1)			(10)
Hemorrhage	2 (20%)	1 (10%)					1 (10%)
Integumentary System							
None							
Musculoskeletal System							
Bone	(10)	(10)	(10)	(9)	(10)	(10)	(10)
Metaphysis, atrophy							10 (100%)
Nervous System							
None							
Respiratory System							
Lung	(10)	(10)	(1)				(10)
Alveolar epithelium, hyperplasia, focal	1 (10%)						
Interstitium, inflammation, chronic	1 (10%)	2 (20%)					2 (20%)

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Special Senses System							
None							
Urinary System							
Kidney	(10)	(10)					(10)
Mineralization, focal							1 (10%)
Nephropathy, chronic	4 (40%)	5 (50%)					
Bilateral, nephropathy, chronic	1 (10%)	2 (20%)					
Bilateral, pelvis, dilatation							1 (10%)
Bilateral, renal tubule, degeneration							2 (20%)
Bilateral, renal tubule, dilatation							3 (30%)
Renal tubule, dilatation	1 (10%)						

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Survivors							
Died last week of study						1	
Terminal sacrifice	10	10	10	10	10	9	10
Animals examined microscopically	10	10	10	10	10	10	10
Alimentary System							
Intestine small, ileum	(10)	(10)				(1)	(10)
Inflammation, chronic		1 (10%)					
Liver	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Basophilic focus						1 (10%)	
Clear cell focus						2 (20%)	3 (30%)
Eosinophilic focus						4 (40%)	2 (20%)
Hepatodiaphragmatic nodule	2 (20%)	3 (30%)	3 (30%)	4 (40%)		2 (20%)	3 (30%)
Inflammation, chronic				5 (50%)	6 (60%)		
Mixed cell focus						8 (80%)	1 (10%)
Pigmentation						10 (100%)	10 (100%)
Bile duct, hyperplasia						5 (50%)	10 (100%)
Hepatocyte, hypertrophy					4 (40%)	10 (100%)	10 (100%)
Hepatocyte, mitotic alteration						3 (30%)	10 (100%)
Hepatocyte, mixed cell focus							1 (10%)
Hepatocyte, necrosis					1 (10%)	7 (70%)	10 (100%)
Hepatocyte, vacuolization cytoplasmic				10 (100%)	10 (100%)	4 (40%)	
Stomach, forestomach	(10)	(10)				(1)	(10)
Edema							1 (10%)
Cardiovascular System							
Heart	(10)	(10)				(1)	(10)
Cardiomyopathy	2 (20%)	1 (10%)					
Endocrine System							
Islets, pancreatic	(10)	(10)				(1)	(10)
Metaplasia							1 (10%)
Thyroid gland	(10)	(10)				(1)	(10)
Ultimobranchial cyst	2 (20%)						
General Body System							
None							

[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 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Genital System							
Clitoral gland	(10)	(9)	(7)	(7)	(10)	(10)	(10)
Atrophy						1 (10%)	7 (70%)
Inflammation, chronic	4 (40%)	1 (11%)					3 (30%)
Inflammation, chronic active			1 (14%)	1 (14%)	4 (40%)	5 (50%)	
Bilateral, inflammation, chronic		1 (11%)					2 (20%)
Bilateral, inflammation, chronic active	1 (10%)						
Ovary	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Cyst		1 (10%)		1 (10%)	3 (30%)		
Interstitial cell, cytoplasmic alteration						3 (30%)	10 (100%)
Uterus	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy						7 (70%)	9 (90%)
Hydrometra	3 (30%)	1 (10%)	2 (20%)	4 (40%)	4 (40%)	1 (10%)	
Hematopoietic System							
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy						4 (40%)	7 (70%)
Lymph node, mandibular	(10)	(10)				(1)	(10)
Hemorrhage		1 (10%)					1 (10%)
Hyperplasia, lymphoid		1 (10%)					
Lymph node, mesenteric	(10)	(10)				(1)	(10)
Infiltration cellular, histiocyte	2 (20%)	1 (10%)					
Spleen	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Pigmentation		1 (10%)			4 (40%)	8 (80%)	8 (80%)
Lymphoid follicle, atrophy							3 (30%)
Red pulp, atrophy							9 (90%)
Thymus	(10)	(10)				(1)	(10)
Hemorrhage		3 (30%)					1 (10%)
Integumentary System							
Skin	(10)	(10)				(1)	(10)
Hemorrhage		1 (10%)					
Hair follicle, atrophy							1 (10%)
Musculoskeletal System							
Bone	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Metaphysis, atrophy						9 (90%)	9 (90%)
Nervous System							
None							

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Respiratory System							
Lung	(10)	(10)				(1)	(10)
Interstitium, inflammation, acute							1 (10%)
Interstitium, inflammation, chronic		1 (10%)					
Nose	(10)	(10)				(1)	(10)
Foreign body		1 (10%)					
Inflammation, chronic active		1 (10%)					
Trachea	(10)	(10)				(1)	(10)
Inflammation, chronic		1 (10%)					
Special Senses System							
None							
Urinary System							
Kidney	(10)	(10)				(1)	(10)
Nephropathy, chronic	1 (10%)	1 (10%)					
Bilateral, mineralization	9 (90%)	10 (100%)				1 (100%)	10 (100%)
Bilateral, pelvis, inflammation, chronic		1 (10%)					
Bilateral, renal tubule, degeneration							1 (10%)
Pelvis, inflammation, chronic		1 (10%)					
Pelvis, transitional epithelium, hyperplasia		1 (10%)					
Renal tubule, degeneration							1 (10%)
Urinary bladder	(10)	(10)				(1)	(10)
Inflammation, chronic		1 (10%)					

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	589 ppm	1,120 ppm	2,300 ppm	4,550 ppm	9,100 ppm
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Survivors							
Died last week of study		2			1		2
Terminal sacrifice	10	8	10	10	9	10	8
Animals examined microscopically	10	10	10	10	10	10	10
Alimentary System							
Gallbladder	(10)	(9)					(9)
Inflammation	1 (10%)						
Liver	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, focal	2 (20%)	2 (20%)					1 (10%)
Necrosis, focal		2 (20%)					
Pigmentation, focal					10 (100%)	10 (100%)	8 (80%)
Bile duct, hyperplasia					7 (70%)	9 (90%)	10 (100%)
Hepatocyte, hypertrophy				7 (70%)	10 (100%)	10 (100%)	10 (100%)
Hepatocyte, necrosis				1 (10%)	8 (80%)	8 (80%)	9 (90%)
Stomach, glandular	(10)	(10)					(10)
Cyst	2 (20%)						1 (10%)
Cardiovascular System							
None							
Endocrine System							
Adrenal cortex	(10)	(10)					(10)
Capsule, hyperplasia	1 (10%)	1 (10%)					3 (30%)
Adrenal medulla	(10)	(10)					(10)
Angiectasis	1 (10%)	2 (20%)					1 (10%)
Pituitary gland	(10)	(10)					(9)
Cyst							1 (11%)
General Body System							
None							
Genital System							
Preputial gland	(10)	(10)	(10)	(10)	(8)	(10)	(9)
Atrophy			4 (40%)	2 (20%)		4 (40%)	5 (56%)
Inflammation							1 (11%)

[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 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Untreated Control	Vehicle Control	589 ppm	1,120 ppm	2,300 ppm	4,550 ppm	9,100 ppm
Hematopoietic System							
Lymph node, mandibular	(8)	(10)					(10)
Hemorrhage	1 (13%)	1 (10%)					
Infiltration, cellular, mast cell							1 (10%)
Spleen	(10)	(10)	(10)	(10)	(10)	(8)	(10)
Hematopoietic cell proliferation		1 (10%)					
Red pulp, atrophy						2 (25%)	3 (30%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)	(9)
Atrophy						1 (10%)	1 (11%)
Thymocyte, necrosis							3 (33%)
Integumentary System							
Skin	(10)	(10)	(1)				(10)
Hair follicle, atrophy			1 (100%)				
Musculoskeletal System							
None							
Nervous System							
None							
Respiratory System							
None							
Special Senses System							
None							
Urinary System							
Kidney	(10)	(10)				(1)	(10)
Infiltration cellular, focal, lymphocyte	1 (10%)						

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachlorethane[a]

	Untreated Control	Vehicle Control	589 ppm	1,120 ppm	2,300 ppm	4,550 ppm	9,100 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							
Liver	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, focal	6 (60%)	5 (50%)	2 (20%)	2 (20%)	1 (10%)	2 (20%)	2 (20%)
Necrosis, focal							2 (20%)
Pigmentation, focal				2 (20%)	9 (90%)	8 (80%)	7 (70%)
Bile duct, hyperplasia					8 (80%)	10 (100%)	10 (100%)
Hepatocyte, hypertrophy			2 (20%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Hepatocyte, necrosis					3 (30%)	7 (70%)	4 (40%)
Stomach, forestomach	(10)	(10)					(10)
Hyperplasia		1 (10%)					
Stomach, glandular	(10)	(10)					(10)
Cyst	2 (20%)	2 (20%)					1 (10%)
Cardiovascular System							
None							
Endocrine System							
Adrenal cortex	(10)	(10)					(10)
Capsule, hyperplasia	5 (50%)	6 (60%)					5 (50%)
Adrenal medulla	(10)	(10)					(10)
Angiectasis	1 (10%)						1 (10%)
Pituitary gland	(9)	(7)					(10)
Cyst							1 (10%)
General Body System							
None							
Genital System							
Clitoral gland	(8)	(7)					(7)
Atrophy	3 (38%)	2 (29%)					5 (71%)

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

TABLE A4
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	Untreated Control	Vehicle Control	589 ppm	1,120 ppm	2,300 ppm	4,550 ppm	9,100 ppm
Hematopoietic System							
Lymph node, mandibular	(10)	(10)					(10)
Hemorrhage	1 (10%)	1 (10%)					
Lymph node, mesenteric	(10)	(10)					(10)
Atrophy		2 (20%)					
Spleen	(10)	(10)	(10)	(10)	(10)	(9)	(10)
Pigmentation					1 (10%)		
Hematopoietic cell proliferation							1 (10%)
Red pulp, atrophy							1 (10%)
Thymus	(10)	(10)	(10)	(10)	(10)	(9)	(10)
Atrophy							2 (20%)
Thymocyte, necrosis		2 (20%)					1 (10%)
Integumentary System							
None							
Musculoskeletal System							
None							
Nervous System							
None							
Respiratory System							
Lung	(9)	(10)	(10)	(7)	(9)	(9)	(10)
Infiltration cellular, focal, lymphocyte				1 (14%)			
Special Senses System							
None							
Urinary System							
Kidney	(10)	(10)					(10)
Infiltration cellular, focal, lymphocyte		1 (10%)					

APPENDIX B
CLINICAL PATHOLOGY RESULTS

TABLE B1 Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane B-2
TABLE B2 Clinical Chemistry Data for Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane B-8

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Male							
n							
Day 5	10	10	10	10	10	10	10
Day 21	10	10	10	10	10	10	9
Week 14	10	10	10	10	10	10	10
Hematology							
Automated hematocrit (%)							
Day 5	38.8 ± 0.4	38.8 ± 0.4	37.8 ± 1.1	39.4 ± 0.5	39.3 ± 0.5	40.3 ± 0.8	42.2 ± 0.8**
Day 21	42.3 ± 0.2	41.9 ± 0.2	42.0 ± 0.3	43.0 ± 0.4*	42.4 ± 0.5	44.6 ± 0.3**	45.1 ± 0.6**
Week 14	45.4 ± 0.3	45.2 ± 0.5	44.9 ± 0.4	44.0 ± 0.9	43.3 ± 0.7	43.1 ± 0.6*	39.0 ± 1.1**
Manual hematocrit (%)							
Day 5	43.4 ± 0.4	43.1 ± 0.4	41.9 ± 1.1	43.9 ± 0.6	43.2 ± 0.5	44.4 ± 0.7	47.4 ± 0.7**
Day 21	47.0 ± 0.3	46.6 ± 0.3	46.5 ± 0.3	47.4 ± 0.3	46.3 ± 0.5	48.1 ± 0.3**	48.3 ± 0.7*
Week 14	48.5 ± 0.5	48.5 ± 0.4	48.2 ± 0.5	47.1 ± 1.0	46.5 ± 0.6*	46.2 ± 0.6*	42.2 ± 1.1**
Hemoglobin (g/dL)							
Day 5	14.5 ± 0.1	14.5 ± 0.1	14.1 ± 0.4	14.7 ± 0.2	14.6 ± 0.2	14.9 ± 0.2	15.7 ± 0.3**
Day 21	15.9 ± 0.1	15.7 ± 0.1	15.7 ± 0.1	15.8 ± 0.1	15.4 ± 0.2	16.0 ± 0.1	16.1 ± 0.2
Week 14	15.9 ± 0.1	15.8 ± 0.1	15.6 ± 0.1	15.2 ± 0.3*	14.9 ± 0.1**	14.6 ± 0.1**	13.6 ± 0.3**
Erythrocytes (10^6/µL)							
Day 5	6.58 ± 0.07	6.57 ± 0.06	6.33 ± 0.22	6.64 ± 0.08	6.67 ± 0.08	6.85 ± 0.13	7.26 ± 0.14**
Day 21	7.33 ± 0.04	7.21 ± 0.06	7.10 ± 0.05	7.27 ± 0.06	7.23 ± 0.12	8.00 ± 0.08**	8.79 ± 0.09**
Week 14	8.96 ± 0.06	8.90 ± 0.11	8.66 ± 0.09	8.43 ± 0.17	8.45 ± 0.16	8.71 ± 0.13	8.78 ± 0.21
Reticulocytes (10^6/µL)							
Day 5	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.15 ± 0.01*	0.12 ± 0.01**	0.09 ± 0.01**
Day 21	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.08 ± 0.01*
Week 14	0.10 ± 0.02	0.07 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
Nucleated erythrocytes (10^3/µL)							
Day 5	0.10 ± 0.03	0.06 ± 0.02	0.11 ± 0.06	0.10 ± 0.03	0.02 ± 0.01	0.06 ± 0.02	0.01 ± 0.01
Day 21	0.07 ± 0.03	0.09 ± 0.03	0.08 ± 0.02	0.06 ± 0.03	0.09 ± 0.03	0.04 ± 0.02	0.06 ± 0.03
Week 14	0.05 ± 0.02	0.08 ± 0.03	0.02 ± 0.02	0.12 ± 0.05	0.05 ± 0.02	0.07 ± 0.02	0.13 ± 0.05
Mean cell volume (fL)							
Day 5	59.0 ± 0.2	59.1 ± 0.2	59.9 ± 0.7	59.3 ± 0.2	58.9 ± 0.3	58.9 ± 0.3	58.1 ± 0.2*
Day 21	57.8 ± 0.3	58.2 ± 0.3	59.2 ± 0.4	59.2 ± 0.2	58.6 ± 0.4	55.8 ± 0.3**	51.3 ± 0.3**
Week 14	50.7 ± 0.2	50.7 ± 0.1	51.8 ± 0.3	52.3 ± 0.2	51.3 ± 0.2	49.4 ± 0.2	44.4 ± 0.4**
Mean cell hemoglobin (pg)							
Day 5	22.0 ± 0.2	22.1 ± 0.1	22.4 ± 0.3	22.1 ± 0.2	21.9 ± 0.1	21.8 ± 0.1	21.7 ± 0.1*
Day 21	21.6 ± 0.1	21.8 ± 0.1	22.1 ± 0.2	21.7 ± 0.1	21.4 ± 0.2	20.0 ± 0.1**	18.4 ± 0.1**
Week 14	17.8 ± 0.1	17.7 ± 0.1	18.1 ± 0.1	18.0 ± 0.1	17.7 ± 0.2	16.8 ± 0.1**	15.5 ± 0.2**
Mean cell hemoglobin concentration (g/dL)							
Day 5	37.3 ± 0.3	37.3 ± 0.3	37.3 ± 0.2	37.3 ± 0.2	37.2 ± 0.2	37.0 ± 0.2	37.3 ± 0.2
Day 21	37.4 ± 0.2	37.5 ± 0.2	37.4 ± 0.2	36.7 ± 0.2*	36.5 ± 0.2**	35.8 ± 0.2**	35.8 ± 0.2**
Week 14	35.0 ± 0.2	35.0 ± 0.3	34.8 ± 0.3	34.5 ± 0.2	34.4 ± 0.4	34.0 ± 0.2	34.8 ± 0.4
Platelets (10^3/µL)							
Day 5	950.2 ± 16.2	948.0 ± 41.5	979.3 ± 24.0	927.4 ± 13.5	946.4 ± 12.2	930.3 ± 21.0	890.1 ± 14.9*
Day 21	900.5 ± 14.0	869.8 ± 41.8	886.3 ± 18.9	898.6 ± 22.5	833.4 ± 14.4*	726.6 ± 14.6**	764.7 ± 26.6**
Week 14	688.5 ± 11.6	728.4 ± 12.3	707.0 ± 5.8	727.0 ± 25.2	716.3 ± 9.7	692.8 ± 12.6	773.4 ± 23.2

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Male (continued)							
n							
Day 5	10	10	10	10	10	10	10
Day 21	10	10	10	10	10	10	9
Week 14	10	10	10	10	10	10	10
Hematology (continued)							
Leukocytes (10^3/µL)							
Day 5	8.82 ± 0.39	9.52 ± 0.28	9.93 ± 0.75	9.17 ± 0.41	9.56 ± 0.33	9.51 ± 0.39	10.08 ± 0.49
Day 21	11.09 ± 0.42	10.96 ± 0.59	11.15 ± 0.44	10.75 ± 0.41	9.94 ± 0.67	9.69 ± 0.33	11.61 ± 0.27
Week 14	12.09 ± 0.35	11.07 ± 0.56	11.31 ± 0.49	13.31 ± 0.39	11.41 ± 0.51	8.89 ± 0.40*	9.85 ± 0.42
Segmented neutrophils (10^3/µL)							
Day 5	0.87 ± 0.13	1.37 ± 0.12	1.38 ± 0.16	1.27 ± 0.12	1.31 ± 0.17	1.19 ± 0.20	1.04 ± 0.18
Day 21	1.62 ± 0.24	1.57 ± 0.16	1.79 ± 0.14	1.24 ± 0.13	1.15 ± 0.14	1.19 ± 0.11	1.75 ± 0.18
Week 14	1.44 ± 0.09	1.58 ± 0.14	1.42 ± 0.15	1.59 ± 0.24	1.42 ± 0.15	1.40 ± 0.16	1.52 ± 0.12
Lymphocytes (10^3/µL)							
Day 5	7.85 ± 0.32	8.02 ± 0.21	8.51 ± 0.79	7.73 ± 0.33	8.05 ± 0.23	8.15 ± 0.28	8.89 ± 0.38
Day 21	9.31 ± 0.38	9.13 ± 0.47	9.15 ± 0.42	9.34 ± 0.32	8.66 ± 0.55	8.35 ± 0.31	9.60 ± 0.29
Week 14	10.49 ± 0.37	9.31 ± 0.51	9.65 ± 0.42	11.52 ± 0.24	9.33 ± 0.51	7.32 ± 0.33*	8.31 ± 0.42
Monocytes (10^3/µL)							
Day 5	0.09 ± 0.04	0.07 ± 0.03	0.05 ± 0.02	0.13 ± 0.03	0.11 ± 0.04	0.15 ± 0.05	0.09 ± 0.05
Day 21	0.09 ± 0.03	0.14 ± 0.06	0.11 ± 0.05	0.11 ± 0.04	0.11 ± 0.05	0.13 ± 0.03	0.17 ± 0.05
Week 14	0.12 ± 0.04	0.13 ± 0.03	0.17 ± 0.04	0.20 ± 0.06	0.14 ± 0.06	0.05 ± 0.02	0.02 ± 0.01*
Eosinophils (10^3/µL)							
Day 5	0.03 ± 0.01	0.06 ± 0.03	0.05 ± 0.03	0.07 ± 0.03	0.09 ± 0.02	0.02 ± 0.01	0.06 ± 0.02
Day 21	0.07 ± 0.03	0.16 ± 0.04	0.11 ± 0.04	0.06 ± 0.03	0.03 ± 0.02*	0.03 ± 0.02*	0.09 ± 0.03
Week 14	0.05 ± 0.02	0.06 ± 0.02	0.08 ± 0.03	0.08 ± 0.05	0.21 ± 0.05*	0.12 ± 0.03	0.05 ± 0.02
Clinical Chemistry							
Creatinine (mg/dL)							
Day 5	0.51 ± 0.01	0.57 ± 0.02	0.55 ± 0.02	0.53 ± 0.02	0.53 ± 0.02	0.53 ± 0.02	0.56 ± 0.02
Day 21	0.61 ± 0.01	0.63 ± 0.02	0.62 ± 0.01	0.62 ± 0.01	0.61 ± 0.01	0.59 ± 0.01	0.64 ± 0.02
Week 14	0.70 ± 0.02	0.70 ± 0.02	0.73 ± 0.02	0.71 ± 0.01	0.68 ± 0.01	0.64 ± 0.02*	0.67 ± 0.02
Total protein (g/dL)							
Day 5	5.9 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	5.6 ± 0.1**	5.5 ± 0.1**	5.4 ± 0.1**	5.3 ± 0.0**
Day 21	6.6 ± 0.1	6.7 ± 0.1	6.4 ± 0.1**	6.6 ± 0.1	6.5 ± 0.1*	6.4 ± 0.1**	6.3 ± 0.0**
Week 14	7.4 ± 0.1	7.2 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	6.7 ± 0.1**	6.0 ± 0.1**
Albumin (g/dL)							
Day 5	4.4 ± 0.0	4.4 ± 0.0	4.3 ± 0.0*	4.2 ± 0.0**	4.2 ± 0.1**	4.3 ± 0.0*	4.3 ± 0.0
Day 21	4.7 ± 0.0	4.8 ± 0.0	4.7 ± 0.0	4.9 ± 0.0	5.0 ± 0.1	5.1 ± 0.0**	5.3 ± 0.0**
Week 14	5.2 ± 0.1	5.2 ± 0.0	5.2 ± 0.1	5.3 ± 0.1	5.5 ± 0.1*	5.3 ± 0.1	5.2 ± 0.1
Cholesterol (mg/dL)							
Day 5	78 ± 2	78 ± 1	71 ± 2*	65 ± 2**	63 ± 2**	56 ± 2**	54 ± 2**
Day 21	71 ± 1	72 ± 2	67 ± 2	64 ± 1	57 ± 2**	63 ± 3	70 ± 4
Week 14	72 ± 1	73 ± 2	74 ± 3	76 ± 2	67 ± 2	68 ± 2	65 ± 2*

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Male (continued)							
n							
Day 5	10	10	10	10	10	10	10
Day 21	10	10	10	10	10	10	9
Week 14	10	10	10	10	10	10	10
Clinical Chemistry (continued)							
Alanine aminotransferase (IU/L)							
Day 5	39 ± 2	37 ± 1	39 ± 2	38 ± 1	40 ± 2	49 ± 2**	68 ± 4**
Day 21	48 ± 1	46 ± 2	45 ± 1	46 ± 1	52 ± 2**	91 ± 4**	140 ± 10**
Week 14	46 ± 2	48 ± 2	49 ± 2	53 ± 2	69 ± 3**	115 ± 8**	292 ± 18**
Alkaline phosphatase (IU/L)							
Day 5	642 ± 15	639 ± 11	609 ± 18	638 ± 10	660 ± 16	759 ± 10**	781 ± 26**
Day 21	505 ± 9	521 ± 10	515 ± 14	514 ± 10	526 ± 10	642 ± 18**	587 ± 23**
Week 14	258 ± 5	256 ± 7	260 ± 5	248 ± 5	245 ± 6	353 ± 12**	432 ± 24**
Creatine kinase (IU/L)							
Day 5	233 ± 19	243 ± 24	294 ± 23	274 ± 36	268 ± 28	325 ± 23*	413 ± 21**
Day 21	339 ± 41	352 ± 30	363 ± 57	308 ± 38	377 ± 39	420 ± 50	597 ± 82*
Week 14	264 ± 40	270 ± 63	286 ± 49	271 ± 38	316 ± 36	350 ± 31	293 ± 29
Sorbitol dehydrogenase (IU/L)							
Day 5	17 ± 1	17 ± 1	19 ± 1	17 ± 1	16 ± 1	18 ± 1	24 ± 2*
Day 21	15 ± 1	13 ± 0	13 ± 1	13 ± 1	14 ± 1	28 ± 2**	40 ± 2**
Week 14	24 ± 1	23 ± 1	27 ± 1*	26 ± 2	31 ± 1**	47 ± 2**	74 ± 4**
5′-Nucleotidase (IU/L)							
Day 5	26 ± 1	26 ± 0	27 ± 1	27 ± 0	27 ± 1	28 ± 1	28 ± 1
Day 21	27 ± 0	27 ± 1	28 ± 1	27 ± 0	27 ± 1	34 ± 1**	34 ± 1**
Week 14	32 ± 1	32 ± 1	32 ± 1	29 ± 0	26 ± 1**	34 ± 1	37 ± 1
Bile acids (µmol/L)							
Day 5	41.7 ± 3.8	36.6 ± 2.9	33.0 ± 2.9	47.7 ± 5.5	41.9 ± 6.2	72.7 ± 5.7**	151.0 ± 31.2**
Day 21	30.4 ± 4.8	27.7 ± 4.0	29.7 ± 4.8	34.0 ± 5.8	29.3 ± 4.6	136.4 ± 32.3**	516.2 ± 59.0**
Week 14	33.7 ± 3.2	29.2 ± 2.9	27.5 ± 2.7	27.2 ± 2.7	35.9 ± 3.9	92.0 ± 16.6**	332.4 ± 47.4**
Female							
n							
Day 5	10	10	10	10	10	10	10
Day 21	10	9	10	10	10	10	9
Week 14	10	10	10	10	10	10	10
Hematology							
Automated hematocrit (%)							
Day 5	42.3 ± 0.5	42.4 ± 0.7	42.2 ± 0.5	42.2 ± 0.5	43.3 ± 0.5	44.8 ± 0.3**	46.3 ± 0.8**
Day 21	44.5 ± 0.3	44.7 ± 0.3	45.6 ± 0.3	45.6 ± 0.5	44.0 ± 0.4	45.8 ± 0.4	44.2 ± 0.6
Week 14	43.2 ± 0.6	42.8 ± 0.4	43.2 ± 0.4	42.1 ± 0.4	40.1 ± 0.5**	42.8 ± 0.7	34.7 ± 0.7**
Manual hematocrit (%)							
Day 5	45.1 ± 0.6	45.6 ± 0.8	45.4 ± 0.6	44.6 ± 0.5	46.3 ± 0.6	48.1 ± 0.5*	49.9 ± 0.7**
Day 21	47.3 ± 0.4	47.4 ± 0.3	48.1 ± 0.3	47.4 ± 0.5	46.4 ± 0.5	48.1 ± 0.5	46.0 ± 0.6
Week 14	46.3 ± 0.5	45.8 ± 0.5	45.8 ± 0.3	44.3 ± 0.4*	42.7 ± 0.6**	45.4 ± 0.8	37.7 ± 0.6**

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Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Female (continued)							
n							
Day 5	10	10	10	10	10	10	10
Day 21	10	9	10	10	10	10	9
Week 14	10	10	10	10	10	10	10
Hematology (continued)							
Hemoglobin (g/dL)							
Day 5	15.5 ± 0.2	15.4 ± 0.2	15.5 ± 0.1	15.4 ± 0.2	15.8 ± 0.1	16.5 ± 0.1**	17.0 ± 0.2**
Day 21	16.1 ± 0.1	16.2 ± 0.1	16.4 ± 0.1	16.3 ± 0.2	15.7 ± 0.1*	16.1 ± 0.1	15.8 ± 0.2
Week 14	15.4 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	14.9 ± 0.1	14.2 ± 0.2**	14.5 ± 0.2**	12.5 ± 0.2**
Erythrocytes (10^6/µL)							
Day 5	7.22 ± 0.09	7.22 ± 0.12	7.17 ± 0.09	7.21 ± 0.10	7.35 ± 0.09	7.71 ± 0.05**	8.06 ± 0.14**
Day 21	7.32 ± 0.05	7.38 ± 0.05	7.46 ± 0.05	7.60 ± 0.08*	7.51 ± 0.07*	8.34 ± 0.07**	8.54 ± 0.10**
Week 14	7.83 ± 0.11	7.72 ± 0.08	7.71 ± 0.07	7.54 ± 0.06	7.52 ± 0.09	8.73 ± 0.16**	7.82 ± 0.13
Reticulocytes (10^6/µL)							
Day 5	0.11 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.06 ± 0.01**	0.04 ± 0.01**
Day 21	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Week 14	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Nucleated erythrocytes (10^3/µL)							
Day 5	0.11 ± 0.03	0.06 ± 0.03	0.08 ± 0.04	0.04 ± 0.02	0.12 ± 0.04	0.05 ± 0.02	0.01 ± 0.01
Day 21	0.03 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.07 ± 0.03	0.04 ± 0.03
Week 14	0.10 ± 0.04	0.09 ± 0.03	0.14 ± 0.04	0.08 ± 0.02	0.08 ± 0.02	0.16 ± 0.03	0.60 ± 0.11**
Mean cell volume (fL)							
Day 5	58.6 ± 0.3	58.7 ± 0.3	58.9 ± 0.2	58.5 ± 0.3	58.8 ± 0.2	58.0 ± 0.1*	57.5 ± 0.2**
Day 21	60.8 ± 0.2	60.5 ± 0.4	61.2 ± 0.3	60.0 ± 0.2	58.6 ± 0.3**	55.0 ± 0.2**	51.7 ± 0.3**
Week 14	55.2 ± 0.2	55.4 ± 0.1	56.1 ± 0.1	55.8 ± 0.1	53.3 ± 0.2*	49.0 ± 0.2**	44.4 ± 0.4**
Mean cell hemoglobin (pg)							
Day 5	21.5 ± 0.1	21.3 ± 0.2	21.6 ± 0.1	21.4 ± 0.1	21.5 ± 0.1	21.4 ± 0.1	21.1 ± 0.2
Day 21	22.0 ± 0.1	22.0 ± 0.1	22.1 ± 0.1	21.5 ± 0.1*	21.0 ± 0.2**	19.3 ± 0.1**	18.5 ± 0.1**
Week 14	19.7 ± 0.1	19.7 ± 0.1	19.8 ± 0.1	19.7 ± 0.1	18.9 ± 0.1**	16.6 ± 0.2**	16.0 ± 0.2**
Mean cell hemoglobin concentration (g/dL)							
Day 5	36.7 ± 0.2	36.3 ± 0.3	36.7 ± 0.2	36.6 ± 0.2	36.6 ±0.2	36.9 ± 0.2	36.8 ± 0.3
Day 21	36.3 ± 0.2	36.4 ± 0.2	36.0 ± 0.2	35.8 ± 0.3	35.8 ± 0.2	35.1 ± 0.3*	35.8 ± 0.2
Week 14	35.7 ± 0.3	35.5 ± 0.1	35.3 ± 0.1	35.4 ± 0.2	35.4 ± 0.2	33.9 ± 0.2**	36.0 ± 0.3
Platelets (10^3/µL)							
Day 5	903.8 ± 23.1	900.3 ± 28.9	894.4 ± 23.7	842.0 ± 23.6	877.5 ± 17.1	861.9 ± 28.0	847.1 ± 29.4
Day 21	834.2 ± 19.9	835.1 ± 17.1	813.4 ± 14.7	774.9 ± 26.1*	774.7 ± 15.6*	689.6 ± 15.1**	637.1 ± 19.8**
Week 14	704.9 ± 18.2	742.1 ± 20.4	725.9 ± 12.7	733.9 ± 8.8	727.4 ± 14.2	639.4 ± 9.9**	662.5 ± 19.4**
Leukocytes (10^3/µL)							
Day 5	9.99 ± 0.28	10.18 ± 0.34	10.83 ± 0.49	10.88 ± 0.42	10.01 ± 0.51	10.94 ± 0.62	12.83 ± 0.72*
Day 21	9.20 ± 0.68	9.86 ± 0.50	9.98 ± 0.50	9.75 ± 0.62	8.87 ± 0.70	9.20 ± 0.45	10.83 ± 0.70
Week 14	9.75 ± 0.37	10.72 ± 0.60	9.60 ± 0.31	9.99 ± 0.69	9.55 ± 0.52	9.02 ± 0.30*	8.00 ± 0.68**
Segmented neutrophils (10^3/µL)							
Day 5	0.98 ± 0.12	1.17 ± 0.11	1.08 ± 0.13	1.45 ± 0.29	0.95 ± 0.10	0.81 ± 0.16	1.33 ± 0.18
Day 21	1.26 ± 0.17	1.16 ± 0.09	1.34 ± 0.14	1.09 ± 0.11	1.00 ± 0.17	0.84 ± 0.11	1.43 ± 0.16
Week 14	1.48 ± 0.12	2.05 ± 0.32	1.30 ± 0.09	1.36 ± 0.21	1.23 ± 0.14	1.16 ± 0.09*	1.57 ± 0.18

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Study of 1,1,2,2-Tetrachloroethane

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Female (continued)							
n							
Day 5	10	10	10	10	10	10	10
Day 21	10	9	10	10	10	10	9
Week 14	10	10	10	10	10	10	10
Hematology (continued)							
Lymphocytes (10^3/µL)							
Day 5	8.90 ± 0.27	8.94 ± 0.30	9.58 ± 0.49	9.28 ± 0.28	8.94 ± 0.46	10.01 ± 0.50	11.27 ± 0.64**
Day 21	7.84 ± 0.58	8.51 ± 0.44	8.47 ± 0.44	8.51 ± 0.51	7.81 ± 0.59	8.20 ± 0.38	9.29 ± 0.60
Week 14	8.14 ± 0.31	8.45 ± 0.34	8.20 ± 0.30	8.53 ± 0.60	8.15 ± 0.40	7.89 ± 0.27	6.87 ± 0.46
Monocytes (10^3/µL)							
Day 5	0.06 ± 0.03	0.03 ± 0.02	0.08 ± 0.04	0.08 ± 0.02	0.03 ± 0.03	0.05 ± 0.03	0.13 ± 0.05
Day 21	0.05 ± 0.02	0.10 ± 0.04	0.09 ± 0.04	0.07 ± 0.05	0.04 ± 0.02	0.10 ± 0.04	0.07 ± 0.03
Week 14	0.08 ± 0.02	0.08 ± 0.03	0.10 ± 0.03	0.07 ± 0.03	0.08 ± 0.04	0.08 ± 0.03	0.09 ± 0.04
Eosinophils (10^3/µL)							
Day 5	0.07 ± 0.03	0.04 ± 0.02	0.12 ± 0.02*	0.08 ± 0.04	0.12 ± 0.04	0.07 ± 0.02	0.10 ± 0.03
Day 21	0.05 ± 0.03	0.09 ± 0.02	0.08 ± 0.03	0.08 ± 0.05	0.04 ± 0.03	0.10 ± 0.03	0.07 ± 0.03
Week 14	0.09 ± 0.02	0.14 ± 0.04	0.07 ± 0.03	0.03 ± 0.02	0.09 ± 0.03	0.13 ± 0.04	0.05 ± 0.02
Clinical Chemistry							
Creatinine (mg/dL)							
Day 5	0.58 ± 0.01	0.58 ± 0.01	0.56 ± 0.02	0.57 ± 0.03	0.59 ± 0.01	0.58 ± 0.02	0.59 ± 0.01
Day 21	0.62 ± 0.02	0.64 ± 0.02	0.64 ± 0.02	0.63 ± 0.02	0.60 ± 0.02	0.61 ± 0.02	0.63 ± 0.02
Week 14	0.70 ± 0.02	0.72 ± 0.01	0.71 ± 0.02	0.71 ± 0.02	0.68 ± 0.01	0.63 ± 0.02**	0.62 ± 0.02**
Total protein (g/dL)							
Day 5	5.8 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.6 ± 0.0	5.7 ± 0.1	5.5 ± 0.1**
Day 21	6.4 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.2 ± 0.1
Week 14	7.1 ± 0.1	7.2 ± 0.1	7.3 ± 0.0	7.3 ± 0.1	6.9 ± 0.1	6.4 ± 0.1**	5.6 ± 0.1**
Albumin (g/dL)							
Day 5	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.0	4.3 ± 0.0	4.5 ± 0.0	4.5 ± 0.1	4.6 ± 0.1*
Day 21	4.7 ± 0.0	4.8 ± 0.1	4.9 ± 0.1	5.0 ± 0.1**	5.1 ± 0.1**	5.3 ± 0.1**	5.3 ± 0.1**
Week 14	5.2 ± 0.1	5.3 ± 0.1	5.5 ± 0.1	5.5 ± 0.0	5.4 ± 0.1	5.4 ± 0.1	4.9 ± 0.1*
Cholesterol (mg/dL)							
Day 5	106 ± 2	108 ± 4	93 ± 1**	91 ± 3**	85 ± 2**	77 ± 2**	64 ± 2**
Day 21	98 ± 2	96 ± 2	92 ± 1	89 ± 2**	69 ± 2**	71 ± 2**	64 ± 3**
Week 14	99 ± 2	104 ± 4	105 ± 3	98 ± 1	81 ± 2**	64 ± 3**	55 ± 3**
Alanine aminotransferase (IU/L)							
Day 5	35 ± 1	32 ± 1	34 ± 1	32 ± 1	41 ± 2**	54 ± 3**	69 ± 4**
Day 21	41 ± 2	37 ± 1	37 ± 2	41 ± 2	46 ± 2**	93 ± 5**	132 ± 7**
Week 14	47 ± 2	46 ± 2	42 ± 1	41 ± 2	49 ± 2	112 ± 7**	339 ± 18**
Alkaline phosphatase (IU/L)							
Day 5	519 ± 12	510 ± 8	510 ± 13	492 ± 11	556 ± 14*	620 ± 16**	616 ± 18**
Day 21	412 ± 12	432 ± 14	389 ± 15	382 ± 7	432 ± 12	545 ± 22*	453 ± 12
Week 14	236 ± 7	227 ± 5	216 ± 4	220 ± 3	225 ± 11	341 ± 7**	468 ± 22**
Creatine kinase (IU/L)							
Day 5	339 ± 36	283 ± 27	372 ± 30	319 ± 38	352 ± 28	382 ± 44	443 ± 31**
Day 21	308 ± 47	256 ± 26	316 ± 34	320 ± 33	448 ± 33**	352 ± 24**	619 ± 68**
Week 14	250 ± 16	237 ± 29	243 ± 16	313 ± 37	347 ± 46	386 ± 45*	290 ± 31

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
Female (continued)							
n							
Day 5	10	10	10	10	10	10	10
Day 21	10	9	10	10	10	10	9
Week 14	10	10	10	10	10	10	10
Clinical Chemistry (continued)							
Sorbitol dehydrogenase (IU/L)							
Day 5	17 ± 1	16 ± 1	19 ± 1*	16 ± 1	16 ± 1	19 ± 2	24 ± 2**
Day 21	15 ± 1	14 ± 1	15 ± 1	16 ± 1*	16 ± 1	34 ± 2**	41 ± 3**
Week 14	28 ± 1	27 ± 1	27 ± 1	28 ± 2	25 ± 1	45 ± 3**	82 ± 3**
5′-Nucleotidase (IU/L)							
Day 5	34 ± 1	33 ± 1	37 ± 1**	38 ± 1**	39 ± 1**	42 ± 1**	40 ± 2**
Day 21	35 ± 1	35 ± 1	37 ± 1	40 ± 1	46 ± 2**	44 ± 2**	35 ± 2
Week 14	35 ± 1	37 ± 1	38 ± 1	42 ± 1*	44 ± 2**	39 ± 1	36 ± 1
Bile acids (µmol/L)							
Day 5	36.1 ± 7.2	24.7 ± 3.6	26.2 ± 3.5	25.4 ± 2.4	30.8 ± 3.5	51.6 ± 5.0**	102.9 ± 15.5**
Day 21	25.9 ± 2.6	34.3 ± 3.6	21.3 ± 1.6	22.5 ± 4.0	29.3 ± 3.4	68.7 ± 10.2*	330.1 ± 52.3**
Week 14	44.5 ± 7.0	37.0 ± 7.1	46.6 ± 6.5	39.1 ± 5.6	36.3 ± 3.9	39.3 ± 7.9	321.5 ± 50.6**

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

TABLE B2
Clinical Chemistry Data for Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	589 ppm	1,120 ppm	2,300 ppm	4,550 ppm	9,100 ppm
n	10	10	10	10	10	10	10
Male							
Creatinine (mg/dL)	0.45 ± 0.02	0.44 ± 0.02	0.44 ± 0.02	0.45 ± 0.02	0.42 ± 0.01	0.46 ± 0.02	0.46 ± 0.02
Total protein (g/dL)	5.3 ± 0.0	5.4 ± 0.1	5.2 ± 0.1	5.1 ± 0.1**	5.1 ± 0.1**	5.1 ± 0.1*	5.1 ± 0.1**
Albumin (g/dL)	3.7 ± 0.0	3.8 ± 0.0	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.0	3.8 ± 0.0	3.9 ± 0.1
Cholesterol (mg/dL)	123 ± 3	131 ± 7	125 ± 4	94 ± 3**	110 ± 5	112 ± 4	126 ± 5
Alanine aminotransferase (IU/L)	89 ± 23	66 ± 8	62 ± 19	74 ± 8	207 ± 18**	172 ± 18**	296 ± 24**
Alkaline phosphatase (IU/L)	85 ± 1	85 ± 2	78 ± 2	89 ± 2	130 ± 3**	143 ± 7**	184 ± 11**
Creatine kinase (IU/L)	530 ± 108[b]	326 ± 73[c]	575 ± 162	236 ± 48[b]	539 ± 130	443 ± 91	453 ± 61[b]
Sorbitol dehydrogenase (IU/L)	55 ± 2	55 ± 3	53 ± 2	76 ± 3**	288 ± 20**	288 ± 29**	448 ± 25**
5′-Nucleotidase (IU/L)	18 ± 1	18 ± 1	16 ± 1	18 ± 0	30 ± 2**	37 ± 3**	62 ± 7**
Bile acids (µmol/L)	24.8 ± 1.0	25.3 ± 1.2	22.8 ± 1.5	24.8 ± 0.6	56.5 ± 5.1**	63.3 ± 7.5**	108.7 ± 8.1**
Female							
Creatinine (mg/dL)	0.49 ± 0.02	0.49 ± 0.02	0.46 ± 0.02	0.48 ± 0.03	0.47 ± 0.02	0.47 ± 0.02	0.49 ± 0.02
Total protein (g/dL)	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.0	5.4 ± 0.1*	5.4 ± 0.0**	5.1 ± 0.1**
Albumin (g/dL)	4.3 ± 0.0	4.3 ± 0.1	4.5 ± 0.1	4.4 ± 0.0	4.3 ± 0.1	4.3 ± 0.0	4.0 ± 0.0**
Cholesterol (mg/dL)	108 ± 3	109 ± 2	109 ± 3	85 ± 3**	68 ± 2**	64 ± 3**	92 ± 4**
Alanine aminotransferase (IU/L)	52 ± 12	34 ± 5	50 ± 15	65 ± 5**	189 ± 33**	197 ± 21**	351 ± 35**
Alkaline phosphatase (IU/L)	134 ± 4	131 ± 5	126 ± 2	139 ± 5	150 ± 3**	161 ± 7**	195 ± 6**
Creatine kinase (IU/L)	490 ± 75[b]	405 ± 53	499 ± 51[b]	423 ± 48[b]	717 ± 116[b]	434 ± 60	570 ± 23[c]
Sorbitol dehydrogenase (IU/L)	41 ± 2	36 ± 1	44 ± 3*	76 ± 4**	197 ± 15**	243 ± 23**	461 ± 59**
5′-Nucleotidase (IU/L)	59 ± 2	59 ± 3	71 ± 2	84 ± 5**	62 ± 2	62 ± 3	83 ± 4**
Bile acids (µmol/L)	28.0 ± 1.1	27.2 ± 1.2	26.1 ± 1.9	30.9 ± 1.1*	44.2 ± 3.9**	51.5 ± 3.6**	101.7 ± 12.0**

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

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 15-Day Feed Study of 1,1,2,2-Tetrachloroethane** **C-2**
TABLE C2 **Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane** **C-3**
TABLE C3 **Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 15-Day Feed Study of 1,1,2,2-Tetrachloroethane** **C-4**
TABLE C4 **Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane** **C-5**

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 15-Day Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	3,325 ppm	6,650 ppm	13,300 ppm
n	5	5	5	5	5
Male					
Necropsy body wt	232 ± 5	235 ± 5	168 ± 8**	125 ± 3**	108 ± 2**
Heart					
Absolute	0.934 ± 0.023	0.895 ± 0.032	0.637 ± 0.039**	0.621 ± 0.053**	0.516 ± 0.040**
Relative	4.03 ± 0.03	3.81 ± 0.09	3.78 ± 0.08	4.98 ± 0.36**	4.77 ± 0.33*
R. Kidney					
Absolute	1.168 ± 0.018	1.159 ± 0.016	0.965 ± 0.029**	0.792 ± 0.034**	0.641 ± 0.014**
Relative	5.04 ± 0.14	4.95 ± 0.09	5.77 ± 0.21**	6.35 ± 0.11**	5.95 ± 0.15**
Liver					
Absolute	14.190 ± 0.313	13.929 ± 0.529	11.056 ± 0.627**	7.187 ± 0.091**	5.648 ± 0.271**
Relative	61.23 ± 1.46	59.38 ± 2.01	65.63 ± 1.11*	57.83 ± 1.15	52.29 ± 2.08*
Lung					
Absolute	1.890 ± 0.149	1.678 ± 0.126	1.232 ± 0.101**	1.096 ± 0.107**	0.840 ± 0.051**
Relative	8.12 ± 0.53	7.18 ± 0.60	7.30 ± 0.39	8.76 ± 0.64	7.76 ± 0.32
R. Testis					
Absolute	1.244 ± 0.032	1.240 ± 0.016	1.137 ± 0.042*	1.063 ± 0.043**	0.969 ± 0.023**
Relative	5.36 ± 0.11	5.29 ± 0.07	6.79 ± 0.23**	8.53 ± 0.17**	8.98 ± 0.08**
Thymus					
Absolute	0.504 ± 0.028	0.535 ± 0.050	0.382 ± 0.052**	0.155 ± 0.010**	0.081 ± 0.005**
Relative	2.17 ± 0.10	2.27 ± 0.16	2.28 ± 0.33	1.25 ± 0.11**	0.75 ± 0.06**
Female					
Necropsy body wt	152 ± 3	152 ± 2	114 ± 2**	95 ± 1**	81 ± 2**
Heart					
Absolute	0.644 ± 0.016	0.619 ± 0.027	0.499 ± 0.026**	0.419 ± 0.008**	0.407 ± 0.015**
Relative	4.24 ± 0.08	4.07 ± 0.19	4.38 ± 0.15	4.39 ± 0.08	5.04 ± 0.24**
R. Kidney					
Absolute	0.763 ± 0.029	0.747 ± 0.025	0.643 ± 0.058*	0.560 ± 0.018**	0.555 ± 0.012**
Relative	5.03 ± 0.11	4.91 ± 0.16	5.63 ± 0.41	5.87 ± 0.14*	6.86 ± 0.18**
Liver					
Absolute	7.007 ± 0.232	7.406 ± 0.206	7.024 ± 0.326	5.251 ± 0.189**	4.602 ± 0.118**
Relative	46.14 ± 0.81	48.76 ± 1.84	61.73 ± 1.95**	54.96 ± 1.33	56.90 ± 1.63*
Lung					
Absolute	1.200 ± 0.078	1.253 ± 0.047[b]	0.906 ± 0.057**	0.886 ± 0.047**	0.824 ± 0.041**
Relative	7.91 ± 0.50	8.16 ± 0.22[b]	7.96 ± 0.43	9.29 ± 0.49	10.17 ± 0.41**
Thymus					
Absolute	0.381 ± 0.029	0.395 ± 0.040	0.239 ± 0.021**	0.148 ± 0.011**	0.071 ± 0.009**
Relative	2.51 ± 0.15	2.61 ± 0.29	2.10 ± 0.18	1.56 ± 0.13**	0.88 ± 0.13**

* 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). All rats in the 26,600 and 53,200 ppm groups died before the end of the study.

[b] n=4

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	268 ppm	589 ppm	1,180 ppm	2,300 ppm	4,600 ppm
n	10	10	10	10	10	10	10
Male							
Necropsy body wt	357 ± 10	366 ± 5	354 ± 9	353 ± 6	341 ± 6*	259 ± 9**	127 ± 5**
Heart							
Absolute	1.018 ± 0.029	1.051 ± 0.020	1.009 ± 0.024	1.028 ± 0.019	0.998 ± 0.016	0.818 ± 0.025**	0.485 ± 0.012**
Relative	2.85 ± 0.03	2.87 ± 0.04	2.85 ± 0.02	2.92 ± 0.03	2.93 ± 0.02	3.16 ± 0.04**	3.85 ± 0.08**
R. Kidney							
Absolute	1.221 ± 0.038	1.241 ± 0.023	1.238 ± 0.017	1.248 ± 0.025	1.286 ± 0.038	1.075 ± 0.025**	0.755 ± 0.032**
Relative	3.42 ± 0.04	3.39 ± 0.04	3.51 ± 0.07	3.54 ± 0.03	3.77 ± 0.05*	4.16 ± 0.07**	5.99 ± 0.21**
Liver							
Absolute	12.455 ± 0.464	12.738 ± 0.259	12.993 ± 0.350	14.473 ± 0.439	15.544 ± 0.396	11.602 ± 0.437*	6.570 ± 0.179**
Relative	34.81 ± 0.45	34.79 ± 0.42	36.72 ± 0.44	41.03 ± 0.85**	45.61 ± 0.52**	44.68 ± 0.45**	52.23 ± 1.42**
Lung							
Absolute	1.615 ± 0.058	1.640 ± 0.058	1.770 ± 0.095	1.615 ± 0.056	1.569 ± 0.051	1.279 ± 0.064**	0.752 ± 0.027**
Relative	4.53 ± 0.12	4.49 ± 0.16	4.98 ± 0.19	4.58 ± 0.14	4.64 ± 0.21	4.91 ± 0.12	5.97 ± 0.19**
R. Testis							
Absolute	1.452 ± 0.035	1.477 ± 0.020	1.469 ± 0.013	1.478 ± 0.021	1.484 ± 0.034	1.393 ± 0.044	0.681 ± 0.073**
Relative	4.08 ± 0.05	4.04 ± 0.05	4.17 ± 0.10	4.20 ± 0.08	4.36 ± 0.07	5.39 ± 0.17**	5.30 ± 0.43**
Thymus							
Absolute	0.365 ± 0.015	0.348 ± 0.020	0.347 ± 0.016	0.350 ± 0.012	0.322 ± 0.012	0.271 ± 0.016**	0.113 ± 0.008**
Relative	1.03 ± 0.04	0.95 ± 0.05	0.98 ± 0.04	1.00 ± 0.04	0.94 ± 0.02	1.04 ± 0.04	0.89 ± 0.05
Female							
Necropsy body wt	193 ± 6	195 ± 4	192 ± 4	189 ± 2	177 ± 2**	139 ± 4**	85 ± 3**
Heart							
Absolute	0.643 ± 0.013	0.651 ± 0.014	0.624 ± 0.013	0.638 ± 0.011	0.588 ± 0.009**	0.501 ± 0.010**	0.352 ± 0.008**
Relative	3.34 ± 0.04	3.34 ± 0.05	3.25 ± 0.04	3.38 ± 0.03	3.33 ± 0.04	3.62 ± 0.05**	4.16 ± 0.09**
R. Kidney							
Absolute	0.691 ± 0.026	0.716 ± 0.015	0.723 ± 0.015	0.715 ± 0.013	0.687 ± 0.014	0.658 ± 0.019**	0.511 ± 0.012**
Relative	3.58 ± 0.05	3.68 ± 0.07	3.77 ± 0.04	3.79 ± 0.03	3.89 ± 0.05	4.74 ± 0.07**	6.05 ± 0.18**
Liver							
Absolute	6.509 ± 0.211	6.839 ± 0.174	7.034 ± 0.125	7.141 ± 0.159	7.801 ± 0.080**	6.660 ± 0.215	4.944 ± 0.121**
Relative	33.78 ± 0.44	35.07 ± 0.56	36.69 ± 0.36	37.84 ± 0.51*	44.20 ± 0.27**	48.03 ± 0.89**	58.40 ± 1.42**
Lung							
Absolute	1.094 ± 0.036	1.125 ± 0.024	1.151 ± 0.038	1.137 ± 0.056	0.989 ± 0.020**	0.823 ± 0.029**	0.602 ± 0.017**
Relative	5.68 ± 0.12	5.77 ± 0.10	5.99 ± 0.12	6.01 ± 0.23	5.60 ± 0.07	5.93 ± 0.10	7.10 ± 0.18**
Thymus							
Absolute	0.267 ± 0.014	0.259 ± 0.015	0.257 ± 0.010	0.258 ± 0.010	0.241 ± 0.008	0.183 ± 0.005**	0.089 ± 0.007**
Relative	1.38 ± 0.03	1.33 ± 0.07	1.34 ± 0.04	1.36 ± 0.05	1.36 ± 0.04	1.32 ± 0.03	1.03 ± 0.05**

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

TABLE C3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 15-Day Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	3,325 ppm	6,650 ppm	13,300 ppm	26,600 ppm
Male						
n	5	5	5	5	3	
Necropsy body wt	24.0 ± 0.6	23.4 ± 0.2	20.3 ± 0.9**	19.0 ± 0.4**	19.1 ± 0.6**	
Heart						
Absolute	0.162 ± 0.007	0.161 ± 0.011	0.130 ± 0.007*	0.131 ± 0.006*	0.112 ± 0.007**	
Relative	6.74 ± 0.22	6.87 ± 0.52	6.41 ± 0.11	6.93 ± 0.32	5.83 ± 0.27	
R. Kidney						
Absolute	0.260 ± 0.015	0.271 ± 0.018	0.199 ± 0.010**	0.225 ± 0.013	0.217 ± 0.019	
Relative	10.79 ± 0.42	11.54 ± 0.70	9.83 ± 0.20	11.89 ± 0.83	11.30 ± 0.65	
Liver						
Absolute	1.435 ± 0.119	1.295 ± 0.077	1.469 ± 0.095	1.475 ± 0.121	1.524 ± 0.118	
Relative	59.41 ± 3.79	55.26 ± 3.30	72.22 ± 1.98*	77.72 ± 6.35**	79.51 ± 4.71**	
Lung						
Absolute	0.254 ± 0.032	0.276 ± 0.017	0.265 ± 0.016	0.308 ± 0.028	0.207 ± 0.020	
Relative	10.47 ± 1.15	11.77 ± 0.72	13.13 ± 0.87	16.36 ± 1.76*	10.78 ± 0.73	
R. Testis						
Absolute	0.110 ± 0.006	0.110 ± 0.004	0.093 ± 0.004*	0.097 ± 0.003	0.091 ± 0.008*	
Relative	4.55 ± 0.14	4.68 ± 0.15	4.61 ± 0.20	5.10 ± 0.19	4.72 ± 0.33	
Thymus						
Absolute	0.054 ± 0.004	0.050 ± 0.005	0.021 ± 0.006	0.020 ± 0.003	0.054 ± 0.032	
Relative	2.25 ± 0.12	2.15 ± 0.22	1.06 ± 0.29	1.02 ± 0.15	2.76 ± 1.57	
Female						
n	5	5	5	5	5	5
Necropsy body wt	20.3 ± 0.5	20.4 ± 0.4	18.2 ± 0.4**	16.3 ± 0.3**	14.2 ± 0.3**	13.4 ± 0.3**
Heart						
Absolute	0.117 ± 0.005	0.127 ± 0.004	0.109 ± 0.007*	0.097 ± 0.003**	0.093 ± 0.003**	0.087 ± 0.003**
Relative	5.77 ± 0.20	6.21 ± 0.27	6.02 ± 0.39	5.96 ± 0.29	6.54 ± 0.15	6.46 ± 0.19
R. Kidney						
Absolute	0.193 ± 0.010	0.198 ± 0.008	0.181 ± 0.008	0.165 ± 0.004**	0.164 ± 0.005**	0.173 ± 0.004*
Relative	9.49 ± 0.36	9.66 ± 0.25	9.91 ± 0.30	10.12 ± 0.38	11.59 ± 0.26**	12.92 ± 0.23**
Liver						
Absolute	1.130 ± 0.073	1.334 ± 0.037	1.293 ± 0.049	1.001 ± 0.041**	1.028 ± 0.028**	1.072 ± 0.030**
Relative	55.63 ± 2.73	65.28 ± 1.64	71.14 ± 3.00	61.39 ± 2.83	72.56 ± 2.05*	80.01 ± 1.35**
Lung						
Absolute	0.226 ± 0.024	0.287 ± 0.030	0.253 ± 0.026	0.211 ± 0.045	0.188 ± 0.016	0.210 ± 0.022
Relative	11.15 ± 1.10	14.11 ± 1.69	13.95 ± 1.53	13.02 ± 2.86	13.26 ± 1.03	15.74 ± 1.78
Thymus						
Absolute	0.085 ± 0.002	0.093 ± 0.003	0.059 ± 0.005**	0.045 ± 0.005**	0.015 ± 0.001**	0.016 ± 0.003**
Relative	4.17 ± 0.10	4.56 ± 0.18	3.25 ± 0.25**	2.79 ± 0.30**	1.08 ± 0.09**	1.16 ± 0.21**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). All male mice in 26,600 ppm group and all mice in the 53,200 ppm groups died before the end of the study.

TABLE C4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	589 ppm	1,120 ppm	2,300 ppm	4,550 ppm	9,100 ppm
n	10	10	10	10	10	10	10
Male							
Necropsy body wt	30.4 ± 0.8	30.1 ± 0.6	30.6 ± 0.6	30.0 ± 0.3	26.5 ± 0.4**	25.2 ± 0.2**	23.1 ± 0.5**
Heart							
Absolute	0.151 ± 0.006	0.156 ± 0.006	0.147 ± 0.003	0.149 ± 0.003	0.128 ± 0.002**	0.125 ± 0.002**	0.116 ± 0.002**
Relative	4.99 ± 0.15	5.18 ± 0.19	4.81 ± 0.11	4.97 ± 0.07	4.83 ± 0.10	4.95 ± 0.06	5.00 ± 0.06
R. Kidney							
Absolute	0.292 ± 0.009	0.292 ± 0.005	0.278 ± 0.008	0.276 ± 0.006	0.228 ± 0.006**	0.208 ± 0.005**	0.207 ± 0.003**
Relative	9.64 ± 0.19	9.74 ± 0.30	9.08 ± 0.24	9.23 ± 0.13	8.61 ± 0.23**	8.26 ± 0.18**	8.97 ± 0.24**
Liver							
Absolute	1.463 ± 0.047	1.467 ± 0.020	1.557 ± 0.039	1.701 ± 0.020**	1.607 ± 0.038*	1.531 ± 0.052	1.558 ± 0.045
Relative	48.13 ± 0.52	48.84 ± 1.17	50.94 ± 0.93	56.82 ± 0.63**	60.63 ± 1.20**	60.71 ± 1.76**	67.43 ± 1.83**
Lung							
Absolute	0.212 ± 0.010	0.225 ± 0.023	0.193 ± 0.005	0.205 ± 0.013	0.175 ± 0.005**	0.176 ± 0.005**	0.195 ± 0.012**
Relative	7.04 ± 0.44	7.46 ± 0.70	6.35 ± 0.23	6.82 ± 0.37	6.61 ± 0.17	7.00 ± 0.16	8.50 ± 0.65
R. Testis							
Absolute	0.124 ± 0.003	0.125 ± 0.003	0.122 ± 0.002	0.123 ± 0.002	0.114 ± 0.002	0.121 ± 0.005	0.110 ± 0.002**
Relative	4.08 ± 0.07	4.14 ± 0.10	3.99 ± 0.07	4.09 ± 0.06	4.30 ± 0.08	4.80 ± 0.23**	4.77 ± 0.07**
Thymus							
Absolute	0.039 ± 0.003	0.043 ± 0.003	0.039 ± 0.003	0.037 ± 0.003	0.039 ± 0.002	0.035 ± 0.002	0.026 ± 0.003**
Relative	1.29 ± 0.08	1.42 ± 0.11	1.27 ± 0.09	1.24 ± 0.10	1.46 ± 0.08	1.40 ± 0.08	1.11 ± 0.13
Female							
Necropsy body wt	25.6 ± 0.8	24.3 ± 0.5	24.2 ± 0.2	24.3 ± 0.6	23.3 ± 0.4	21.7 ± 0.2**	21.5 ± 0.6**
Heart							
Absolute	0.124 ± 0.003	0.117 ± 0.003	0.120 ± 0.002	0.127 ± 0.004	0.112 ± 0.003	0.106 ± 0.002*	0.104 ± 0.004**
Relative	4.85 ± 0.09	4.81 ± 0.10	4.97 ± 0.06	5.21 ± 0.19*	4.80 ± 0.08	4.87 ± 0.08	4.81 ± 0.09
R. Kidney							
Absolute	0.181 ± 0.006	0.167 ± 0.004	0.173 ± 0.003	0.174 ± 0.005	0.165 ± 0.004	0.157 ± 0.002	0.167 ± 0.008
Relative	7.07 ± 0.09	6.91 ± 0.16	7.13 ± 0.11	7.15 ± 0.11	7.08 ± 0.10	7.21 ± 0.11	7.77 ± 0.31**
Liver							
Absolute	1.161 ± 0.034	1.048 ± 0.028	1.160 ± 0.022*	1.356 ± 0.058**	1.336 ± 0.037**	1.277 ± 0.030**	1.386 ± 0.047**
Relative	45.37 ± 0.47	43.26 ± 1.05	47.90 ± 0.85**	55.54 ± 1.17**	57.39 ± 0.84**	58.73 ± 1.23**	64.42 ± 1.14**
Lung							
Absolute	0.177 ± 0.006	0.170 ± 0.007	0.164 ± 0.005	0.183 ± 0.013	0.163 ± 0.004	0.161 ± 0.004	0.160 ± 0.006
Relative	6.93 ± 0.25	7.02 ± 0.34	6.79 ± 0.20	7.46 ± 0.34	6.99 ± 0.14	7.43 ± 0.21	7.47 ± 0.22
Thymus							
Absolute	0.048 ± 0.003	0.039 ± 0.003	0.044 ± 0.002	0.042 ± 0.002	0.038 ± 0.002	0.035 ± 0.002	0.033 ± 0.003*
Relative	1.86 ± 0.09	1.63 ± 0.10	1.81 ± 0.07	1.74 ± 0.09	1.64 ± 0.07	1.61 ± 0.08	1.54 ± 0.15

* 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).

APPENDIX D
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE D1	**Summary of Reproductive Tissue Evaluations for Male Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane**	**D-2**
TABLE D2	**Estrous Cycle Characterization for Female Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane**	**D-3**
TABLE D3	**Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane**	**D-4**
TABLE D4	**Estrous Cycle Characterization for Female Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane**	**D-5**

TABLE D1
Summary of Reproductive Tissue Evaluations for Male Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	589 ppm	1,180 ppm	2,300 ppm
n	10	10	10	10	10
Weights (g)					
Necropsy body wt	355 ± 10	366 ± 5	352 ± 6	340 ± 6**	258 ± 9**
L. Cauda epididymis	0.1631 ± 0.0067	0.1660 ± 0.0045[b]	0.1654 ± 0.0038	0.1626 ± 0.0064[b]	0.1221 ± 0.0055**
L. Epididymis	0.4925 ± 0.0103	0.5323 ± 0.0284	0.4981 ± 0.0133	0.4731 ± 0.0116*	0.4038 ± 0.0165**
L. Testis	1.5906 ± 0.0659	1.4405 ± 0.0733	1.5554 ± 0.0480	1.5384 ± 0.0313	1.4183 ± 0.0745
Spermatid measurements					
Spermatid heads (10^7/g testis)	7.86 ± 0.32	9.16 ± 0.73	9.11 ± 0.40	7.61 ± 0.41	9.55 ± 0.29
Spermatid heads (10^7/testis)	12.42 ± 0.56	12.78 ± 0.49	14.06 ± 0.46	11.71 ± 0.68	13.43 ± 0.59
Spermatid count (mean/10^{-4} mL suspension)	62.10 ± 2.78	63.90 ± 2.43	70.28 ± 2.29	58.55 ± 3.42	67.15 ± 2.95
Epididymal spermatozoal measurements					
Motility (%)	81.88 ± 0.98	83.58 ± 0.86	69.30 ± 3.34**	71.09 ± 1.70**	63.49 ± 3.65**
Concentration (10^6/g cauda epididymal tissue)	425 ± 23	330 ± 40	437 ± 73	317 ± 50[b]	194 ± 14

* 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' test (body and tissue weights) or Shirley's test (motility)
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (testis weight) or Dunn's test (spermatid measurements, epididymal spermatozoal concentration).
[b] n=9

TABLE D2
Estrous Cycle Characterization for Female Rats in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	589 ppm	1,180 ppm	2,300 ppm
n	10	10	10	10	10
Necropsy body wt (g)	193 ± 6	195 ± 4	189 ± 2	177 ± 2**	139 ± 4**
Estrous cycle length (days)	5.00 ± 0.00	5.10 ± 0.10	4.95 ± 0.05	5.30 ± 0.20	5.67 ± 0.67[b]
Estrous stages[c] (% of cycle)					
Diestrus	40.8	40.0	44.2	43.3	60.8
Proestrus	16.7	15.0	15.0	18.3	11.7
Estrus	21.7	24.2	21.7	20.0	14.2
Metestrus	20.8	20.8	19.2	18.3	13.3

** 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.

[b] Estrous cycle was longer than 12 days or unclear in 4 of 10 animals.

[c] Evidence shows that females in the 2,300 ppm group differ significantly (Wilk's Criterion, P≤0.05) from the vehicle control group in the relative length of time spent in the estrous stages. Exposed females spent more time in diestrus and less time in proestrus, estrus, and metestrus than did the vehicle control females.

TABLE D3
Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	1,120 ppm	4,550 ppm	9,100 ppm
n	10	10	10	10	10
Weights (g)					
Necropsy body wt	30.4 ± 0.8	30.1 ± 0.6	30.0 ± 0.3	25.2 ± 0.2**	23.1 ± 0.5**
L. Cauda epididymis	0.0189 ± 0.0010	0.0183 ± 0.0011	0.0168 ± 0.0012	0.0160 ± 0.0012	0.0130 ± 0.0007**
L. Epididymis	0.0507 ± 0.0012	0.0480 ± 0.0020	0.0487 ± 0.0018	0.0433 ± 0.0012	0.0410 ± 0.0016**
L. Testis	0.1184 ± 0.0038	0.1150 ± 0.0032	0.1169 ± 0.0018	0.1076 ± 0.0015*	0.1077 ± 0.0027*
Spermatid measurements					
Spermatid heads (10^7/g testis)	16.78 ± 0.92	17.45 ± 0.92	18.22 ± 0.63	17.15 ± 0.69	18.65 ± 0.67
Spermatid heads (10^7/testis)	1.97 ± 0.08	2.01 ± 0.12	2.12 ± 0.06	1.85 ± 0.08	2.00 ± 0.06
Spermatid count (mean/10^{-4} mL suspension)	61.53 ± 2.63	62.70 ± 3.71	66.35 ± 1.80	57.68 ± 2.48	62.50 ± 2.03
Epididymal spermatozoal measurements					
Motility (%)	85.95 ± 0.65	86.46 ± 0.88[b]	86.28 ± 0.59	85.07 ± 0.58[c]	83.81 ± 0.58*[b]
Concentration (10^6/g cauda epididymal tissue)	574 ± 89	567 ± 117	505 ± 82	422 ± 73[b]	374 ± 78

* Significantly different (P≤0.05) from the vehicle control group by Williams' (left testis weight) or Shirley's test (motility)
** 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 for spermatid measurements and epididymal spermatozoal concentration are not significant by Dunn's test.
[b] n=8
[c] n=9

TABLE D4
Estrous Cycle Characterization for Female Mice in the 14-Week Feed Study of 1,1,2,2-Tetrachloroethane[a]

	Untreated Control	Vehicle Control	1,120 ppm	4,550 ppm	9,100 ppm
n	10	10	10	10	10
Necropsy body wt (g)	25.6 ± 0.8	24.3 ± 0.5	24.3 ± 0.6	21.7 ± 0.2**	21.5 ± 0.6**
Estrous cycle length (days)	4.00 ± 0.00[b]	4.50 ± 0.50[b]	4.17 ± 0.12[c]	4.31 ± 0.13[b]	4.89 ± 0.31*[c]
Estrous stages (% of cycle)					
Diestrus	32.5	37.5	29.2	31.7	31.7
Proestrus	10.8	11.7	12.5	15.8	15.8
Estrus	34.2	34.2	35.8	33.3	34.2
Metestrus	22.5	16.7	22.5	19.2	18.3

* Significantly different (P≤0.05) from the vehicle control group by Shirley's test
** 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. By multivariate analysis of variance, exposed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.
[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

APPENDIX E
GENETIC TOXICOLOGY

TABLE E1 Mutagenicity of 1,1,2,2-Tetrachloroethane in *Salmonella typhimurium* E-2

TABLE E2 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,2,2-Tetrachloroethane E-5

TABLE E3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 1,1,2,2-Tetrachloroethane E-10

TABLE E4 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 1,1,2,2-Tetrachloroethane E-11

TABLE E5 Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by 1,1,2,2-Tetrachloroethane E-12

TABLE E6 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of 1,1,2,2-Tetrachloroethane in Feed for 14 Weeks E-13

TABLE E1
Mutagenicity of 1,1,2,2-Tetrachloroethane in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b]					
		–S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Study performed at Case Western Reserve University							
TA100	0	121 ± 13.4	180 ± 17.2	156 ± 7.7	183 ± 6.9	170 ± 8.9	178 ± 4.4
	10	105 ± 3.8	172 ± 7.8	170 ± 2.2	184 ± 7.3	173 ± 10.8	180 ± 2.1
	33	127 ± 4.7	179 ± 5.1	155 ± 6.7	177 ± 2.6	182 ± 3.8	166 ± 15.8
	100	113 ± 9.2	173 ± 2.9	175 ± 7.6	195 ± 8.4	168 ± 10.5	172 ± 11.4
	333	89 ± 8.4	161 ± 14.7	192 ± 4.8	207 ±10.1	198 ± 22.5	189 ± 8.8
	1,000	2 ± 0.9	166 ± 6.7	0 ± 0.0	135 ± 8.4	1 ± 0.6	153 ± 11.8
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		592 ± 3.0	742 ± 24.3	1,904 ± 113.1	1,985 ± 198.7	819 ± 28.7	821 ± 24.2

Strain	Dose (µg/plate)	–S9		+10% hamster S9		
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 3
TA1535	0	6 ± 0.7	6 ± 0.3	9 ± 1.0	5 ± 1.2	10 ± 1.9
	10	5 ± 0.3	4 ± 0.7	12 ± 0.9		11 ± 0.7
	33	8 ± 0.3	7 ± 1.5	16 ± 1.8		12 ± 0.9
	100	6 ± 0.3	5 ± 1.0	18 ± 0.3	4 ± 0.7	13 ± 0.9
	167				5 ± 0.9	
	333	6 ± 0.7	5 ± 1.9	19 ± 0.3	Toxic	15 ± 1.2
	667				0 ± 0.0	
	1,000	7 ± 2.0	6 ± 3.0	Toxic	0 ± 0.0	Toxic
Trial summary		Negative	Negative	Weakly Positive	Negative	Negative
Positive control		378 ± 16.0	261 ± 53.0	99 ± 5.2	99 ± 11.5	97 ± 8.6

Strain	Dose (µg/plate)	+10% rat S9		
		Trial 1	Trial 2	Trial 3
TA1535	0	6 ± 1.3	6 ± 0.6	10 ± 1.3
	10	6 ± 1.8		7 ± 0.9
	33	11 ± 1.2		8 ± 1.2
	100	17 ± 0.9	2 ± 0.6	13 ± 1.2
	167		4 ± 0.9	
	333	17 ± 1.5	7 ± 0.7	17 ± 1.5
	667		1 ± 0.3	
	1,000	Toxic	1 ± 0.6	2 ± 0.3
Trial summary		Equivocal	Negative	Negative
Positive control		45 ± 3.7	36 ± 1.8	72 ± 11.2

TABLE E1
Mutagenicity of 1,1,2,2-Tetrachloroethane in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate					
		–S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Study performed at Case Western Reserve University (continued)							
TA1537	0	5 ± 0.9	6 ± 1.5	8 ± 1.2	11 ± 1.8	9 ± 0.3	16 ± 3.4
	10	4 ± 0.9	6 ± 0.9	8 ± 1.2	13 ± 1.7	7 ± 0.3	19 ± 3.7
	33	5 ± 0.6	9 ± 1.5	6 ± 0.3	10 ± 0.9	5 ± 0.6	12 ± 2.2
	100	2 ± 1.0	7 ± 2.9	7 ± 0.7	14 ± 2.3	9 ± 2.0	18 ± 1.3
	333	2 ± 0.3	8 ± 3.0	4 ± 0.6	12 ± 3.2	8 ± 0.3	15 ± 1.5
	1,000	Toxic	11 ± 1.8	0 ± 0.0	16 ± 2.0	2 ± 1.0	12 ± 3.4
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		900 ± 17.8	790 ± 203.5	175 ± 13.5	371 ± 45.5	53 ± 2.9	128 ± 22.7
TA98	0	19 ± 2.0	26 ± 2.6	21 ± 1.0	30 ± 3.5	24 ± 4.0	38 ± 3.8
	10	14 ± 2.6	26 ± 3.5	22 ± 0.6	37 ± 4.6	20 ± 1.2	43 ± 5.9
	33	23 ± 0.9	31 ± 1.2	20 ± 3.8	31 ± 3.6	26 ± 4.9	43 ± 1.2
	100	17 ± 1.3	27 ± 2.6	21 ± 2.6	34 ± 2.0	19 ± 1.2	36 ± 5.2
	333	19 ± 1.9	27 ± 1.0	25 ± 3.3	38 ± 1.9	16 ± 0.7	36 ± 6.1
	1,000	10 ± 0.3	24 ± 5.0	3 ± 1.0	6 ± 2.8	2 ± 0.5	10 ± 4.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		290 ± 24.2	349 ± 36.7	1,975 ± 45.7	2,285 ± 288.6	412 ± 9.2	533 ± 85.0

Study performed at Inveresk Research International

Strain	Dose (µg/plate)	+10% mouse S9	
		Trial 1	Trial 2
TA100	0	96 ± 5.7	86 ± 2.2
	33	104 ± 2.1	93 ± 0.6
	100	96 ± 3.7	89 ± 4.1
	333	104 ± 2.2	98 ± 2.9
	1,000	94 ± 6.2[d]	81 ± 4.7
	3,333	9 ± 1.2[d]	74 ± 3.3[d]
Trial summary		Negative	Negative
Positive control		250 ± 19.8	281 ± 6.7
TA1535	0	13 ± 2.3	10 ± 2.2
	33	8 ± 0.6	7 ± 2.7
	100	13 ± 2.3	14 ± 0.9
	333	8 ± 1.7	12 ± 1.8
	1,000	5 ± 0.9[d]	11 ± 1.9
	3,333	5 ± 0.6[d]	3 ± 0.3[d]
Trial summary		Negative	Negative
Positive control		52 ± 2.3	42 ± 3.8

TABLE E1
Mutagenicity of 1,1,2,2-Tetrachloroethane in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate +10% mouse S9 Trial 1	Trial 2
Study performed at Inveresk Research International (continued)			
TA97	0	100 ± 3.2	105 ± 3.2
	33	103 ± 1.2	110 ± 4.7
	100	103 ± 4.5	93 ± 7.8
	333	107 ± 0.6	99 ± 8.1
	1,000	72 ± 6.8[d]	94 ± 2.2
	3,333	13 ± 1.7[d]	1 ± 0.6[d]
Trial summary		Negative	Negative
Positive control		178 ± 3.8	200 ± 2.7
TA98	0	16 ± 2.0	20 ± 2.2
	33	16 ± 1.9	23 ± 1.3
	100	16 ± 1.5	25 ± 1.8
	333	15 ± 2.1	21 ± 1.0
	1,000	10 ± 1.2[d]	24 ± 2.1
	3,333	3 ± 3.0[d]	18 ± 3.5[d]
Trial summary		Negative	Negative
Positive control		151 ± 7.8	164 ± 16.6

[a] For the study performed at Case Western Reserve University, the detailed protocol and the data are presented by Haworth *et al.* (1983). The protocol for the Inveresk Research International study was modified from that of Haworth *et al.* (1983). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97 and TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[d] Slight toxicity

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,2,2-Tetrachloroethane[a]

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction
–S9						
Trial 1						
Ethanol[c]		94	106	79	28	
		94	89	71	25	
		96	114	93	32	
		73	92	85	39	31
1,1,2,2-Tetrachloroethane (nL/mL)	60	97	48	86	30	
		84	30	105	42	
		76	14	88	39	37
	80	60	21	79	44	
		54	21	101	63	
		99	30	84	28	45
	100	78	25	98	42	
		67	19	80	40	
		106	33	68	21	34
	120	64	21	88	46	
		87	26	103	40	
		83	23	110	44	43
	150	61	20	94	51	
		58	10	86	49	
		63	13	100	53	51*
	200	93	19	86	31	
		85	18	121	47	
		41	9	57	46	41
Methyl methanesulfonate[d] (µg/mL)	5	38	12	518	458	
		43	24	629	486	
		54	35	691	429	458*

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,2,2-Tetrachloroethane

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
–S9						
Trial 2						
Ethanol		112	92	53	16	
		112	108	51	15	15
1,1,2,2-Tetrachloroethane (nL/mL)	25	76	54	34	15	
		63	53	33	18	
		74	63	35	16	16
	50	58	38	24	14	
		53	28	42	26	
		50	26	25	17	19
	75	59	22	45	25	
		68	25	37	18	
		64	31	38	20	21
	100	72	24	40	19	
		68	16	48	23	
		63	26	42	22	21
	150	59	17	30	17	
		60	18	39	22	
		67	17	40	20	19
	200	73	8	22	10	
		71	6	14	7	
		75	7	22	10	9
	300	Lethal				
		Lethal				
		Lethal				
Methyl methanesulfonate (µg/mL)	5	69	31	311	151	
		56	30	229	136	
		74	33	266	119	135*

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,2,2-Tetrachloroethane

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9						
Trial 1						
Ethanol		110	88	99	30	
		104	70	92	29	
		117	129	70	20	
		117	113	107	31	28
1,1,2,2-Tetrachloroethane (nL/mL)	50	101	110	72	24	
		95	88	110	38	
		111	99	131	39	34
	75	116	102	108	31	
		106	83	113	36	
		102	66	102	33	33
	100	69	73	98	47	
		86	94	90	35	
		103	49	119	38	40
	150	93	68	109	39	
		98	77	79	27	
		114	87	98	29	32
	200	79	52	72	30	
		115	108	56	16	
		94	65	76	27	24
	300	108	112	94	29	
		107	107	68	21	
		111	104	68	20	24
Methylcholanthrene[d] (µg/mL)	2.5	98	25	655	222	
		38	3	416	368	
		117	35	877	251	280*

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,2,2-Tetrachloroethane

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9						
Trial 2						
Ethanol		110	100	172	52	
		89	121	119	45	
		100	68	67	22	
		107	111	184	57	44
1,1,2,2-Tetrachloroethane (nL/mL)	50	116	78	123	35	
		94	63	164	58	
		87	74	226	87	60
	75	113	102	187	55	
		91	93	163	60	
		108	62	115	36	50
	100	100	65	188	63	
		106	79	167	53	
		86	35	157	61	59
	150	89	57	128	48	
		115	76	118	34	
		97	56	62	21	35
	200	104	55	90	29	
		115	26	91	26	
		113	31	95	28	28
	300	111	11	123	37	
		107	11	76	24	30
		Lethal				
	500	Lethal				
		Lethal				
		Lethal				
Methylcholanthrene (µg/mL)	2.5	28	4	438	525	
		51	18	824	537	
		46	7	666	479	513*

TABLE E2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by 1,1,2,2-Tetrachloroethane

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9						
Trial 3						
Ethanol		105	63	107	34	
		105	101	136	43	
		114	136	121	35	37
		Lethal				
1,1,2,2-Tetrachloroethane (nL/mL)	50	107	69	79	25	
		103	91	122	39	
		104	17	94	30	31
	75	100	13	84	28	
		106	76	109	34	31
		Lethal				
	100	107	69	109	34	
		110	73	94	28	
		99	64	95	32	31
	150	111	71	114	34	
		Lethal				
	200	112	65	91	27	
		102	23	106	35	31
		Lethal				
	300	Lethal				
		Lethal				
		Lethal				
Methylcholanthrene (µg/mL)	2.5	114	46	960	281	
		86	24	663	258	269*

* Positive response ($P \leq 0.05$) versus the solvent control
[a] Study was performed at Litton Bionetics, Inc. The detailed protocol is presented by Myhr *et al.* (1985).
[b] Mutant fraction=mutant cells/10^6 clonable cells
[c] Solvent control
[d] Positive control

TABLE E3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 1,1,2,2-Tetrachloroethane[a]

Compound	Concentration (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								
Summary: Positive								
Dimethylsulfoxide[c]		50	1,035	546	0.52	10.9	25.8	
1,1,2,2-Tetrachloroethane	16.8	50	1,035	655	0.63	13.1	25.8	19.96
	55.8	50	1,042	895	0.85	17.9	25.8	62.82*
	168	50	1,030	995	0.96	19.9	31.0[d]	83.12*
					P<0.001[e]			
Mitomycin-C[f]	0.005	25	516	807	1.56	32.3	25.8	196.47
+S9								
Summary: Positive								
Dimethylsulfoxide		50	1,040	505	0.48	10.1	25.5	
1,1,2,2-Tetrachloroethane	451	50	1,037	617	0.59	12.3	25.5	22.53*
	502	50	1,031	645	0.62	12.9	25.5	28.84*
	558[g]	50	1,017	650	0.63	13	25.5	31.62*
					P<0.001			
Cyclophosphamide[f]	1.5	25	518	773	1.49	30.9	25.5	207.32

* Positive response (≥20% increase over solvent control)
[a] Study was performed at Litton Bionetics, Inc. 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] Due to cell cycle delay, harvest time was extended to maximize the number of second-division metaphase cells available for analysis.
[e] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose
[f] Positive control
[g] Precipitate formed at this concentration.

TABLE E4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 1,1,2,2-Tetrachloroethane[a]

	Concentration (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
–S9					
Harvest time: 21.5 hours[b]					
Summary: Negative					
Dimethylsulfoxide[c]		100	1	0.01	1.0
1,1,2,2-Tetrachloroethane	453	79	3	0.04	4.0
	503	100	3	0.03	3.0
	553	—[d]	—	—	—
	603	—	—	—	—
	653	100	6	0.06	6.0
	704	—	—	—	—
	804	—	—	—	—
					P=0.033[e]
Mitomycin-C[f]	0.125	50	47	0.94	44.0*
+S9					
Harvest time: 20.5 hours[b]					
Summary: Negative					
Dimethylsulfoxide		100	3	0.03	3.0
1,1,2,2-Tetrachloroethane	503	100	3	0.03	3.0
	553	100	3	0.03	2.0
	603	100	2	0.02	1.0
	653[g]	41	1	0.02	2.0
					P=0.829
Cyclophosphamide[f]	10	50	34	0.68	46.0*

* Positive response ($P \leq 0.05$) versus the solvent control
[a] Study was performed at Litton Bionetics, Inc. The detailed protocol and these data are presented by Galloway *et al.* (1987).
[b] Due to cell cycle delay, harvest time was extended to maximize the number of first-division metaphase cells available for analysis.
[c] Solvent control
[d] Most cells were dead; no scorable first-division metaphase cells were obtained.
[e] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[f] Positive control
[g] This concentration was extremely toxic; only 41 scorable first-division metaphase cells were found.

TABLE E5
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by 1,1,2,2-Tetrachloroethane[a]

Route of Exposure	Dose (ppm)	Incidence of Death (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested			Total[b]
				Mating 1	Mating 2	Mating 3	
Injection	800	0	0	5/2,024	3/1,910	1/1,753	9/5,687 (0.16%)
	0			4/1,947	0/1,747	3/1,559	7/5,253 (0.13%)
Feed	1,500	1	8	3/1,974	2/1,874	1/1,859	6/5,707 (0.11%)
	0			1/2,224	2/2,136	0/1,824	3/6,184 (0.05%)

[a] Study was performed at the University of Wisconsin, Madison. The detailed protocol and these data are presented by Woodruff *et al.* (1985). The mean mutant frequency from 518 negative control experiments is 0.074% (Mason *et al.*, 1992).

[b] Total number of lethal mutations/total number of X chromosomes tested for three mating trials; total number of lethal mutations/total number of X chromosomes tested by a normal approximation to the binomial test were not significant (Margolin *et al.*, 1983).

TABLE E6
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of 1,1,2,2-Tetrachloroethane in Feed for 14 Weeks[a]

Compound	Exposure Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]
Male				
Vehicle control		5	2.10 ± 0.29	
1,1,2,2-Tetrachloroethane	589	5	2.30 ± 0.25	0.3814
	1,120	5	3.00 ± 0.16	0.1035
	2,300	5	3.30 ± 0.12	0.0510
	4,550	5	4.50 ± 0.27	0.0015
	9,100	5	5.10 ± 0.43	0.0002
			P<0.001[d]	
Female				
Vehicle control		5	2.20 ± 0.25	
1,1,2,2-Tetrachloroethane	589	5	2.50 ± 0.27	0.3307
	1,120	5	2.60 ± 0.19	0.2816
	2,300	5	2.90 ± 0.29	0.1632
	4,550	5	3.40 ± 0.10	0.0542
	9,100	5	3.80 ± 0.34	0.0193
			P=0.008	

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

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF 1,1,2,2-TETRACHLOROETHANE

1,1,2,2-Tetrachloroethane was obtained from Eastman Kodak Company (Rochester, NY) in one lot (B17) for use in the 15-day and 14-week studies. Microencapsulation of the chemical was performed by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the loaded microcapsules were assigned a separate lot number (335-2A). Identity, purity, stability, and water content analyses of the neat and microencapsulated chemical were conducted by the analytical chemistry laboratory and the study laboratories. Reports on analyses performed in support of the 1,1,2,2-tetrachloroethane studies are on file at the National Institute of Environmental Health Sciences.

Analyses of Neat Chemical

The chemical, a clear, colorless liquid, was identified as 1,1,2,2-tetrachloroethane by the analytical chemistry laboratory using infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy; identity was confirmed by the study laboratories using infrared spectroscopy. All spectra were consistent with the literature spectra (*Sadtler Standard Spectra*, 1970) and with the structure of 1,1,2,2-tetrachloroethane. The infrared and nuclear magnetic resonance spectra are presented in Figures F1 and F2.

The purity of lot B17 was determined by elemental analyses and gas chromatography (GC) with flame ionization detection using systems A and B (Table F1). Additional GC analyses with mass spectroscopy (GC/MS) were performed to determine whether selected chlorinated impurities were present and to quantify any impurities that were detected. The 14-week study laboratory analyzed purity using GC by system A.

Elemental analyses for carbon, hydrogen, and chlorine were in agreement with the theoretical values for 1,1,2,2-tetrachloroethane. GC indicated one major peak and one impurity with an area of 0.15% (system A) or 0.13% (system B) relative to the major peak area. GC/MS by systems C through E or similar systems detected trichloroethylene (393 ± 35 ppm) and tetrachloroethylene (13 ± 1 ppm) as impurities and tentatively identified chloroform, *cis*-1,2-dichloroethylene, and *trans*-1,2-dichloroethylene as impurities at concentrations less than 1 ppm. The overall purity of lot B17 was determined by the analytical chemistry laboratory to be greater than 99%. Using GC by system A, the study laboratory confirmed that the purity was 99% or greater; one impurity with an area greater than 0.1% of the total peak area and four minor impurities were detected. Karl Fischer titration indicated 0.014% ± 0.007% water.

Based on the manufacturer's recommendations, the bulk chemical was stored frozen.

Microcapsule Formulation and Analyses

Microcapsules loaded with neat 1,1,2,2-tetrachloroethane and placebos (empty microcapsules) were prepared by the analytical chemistry laboratory with a proprietary process using food-grade, modified corn starch and reagent-grade sucrose (80:20) to produce dry microspheres; the outer surfaces of the microcapsules were dusted with food-grade, hydrophobic, modified corn starch. Following microencapsulation, the analytical chemistry laboratory tested the chemical for conformance to specifications. The microcapsules were examined microscopically for appearance. Conformance to particle size specifications (with no more than 1% of particles having diameters greater than 420 µm) was determined by passing placebo and loaded microcapsules through U.S. standard sieves (numbers 30, 40, 60, 80, 100, and 120). The chemical loads of freshly prepared microcapsules and of microcapsules stored under a variety of conditions were determined with GC by systems F through H. Samples for GC analysis were prepared by extracting the microcapsules (approximately 0.5 g) with 50 mL of a methanol:water (50:50) solution by shaking for 15 minutes; 50 mL of an internal standard solution (0.8 mg/mL chlorobenzene in methanol) were added, and the mixtures were shaken. Comparisons of the impurity profiles of the neat and microencapsulated 1,1,2,2-tetrachloroethane and 4- and 20-month stability studies were also performed with GC by systems F and H.

Microscopic examination of the microcapsules revealed no unusual characteristics. Loaded microcapsules were slightly outside the size specification, with 1.1% having diameters greater than 420 µm; this was not expected to have a significant effect on the studies. The placebo particles were within the size specifications. The mean 1,1,2,2-tetrachloroethane load was 54.0% ± 0.3%. Microcapsules exposed to animal room conditions (50% relative humidity, 25° C) in open dishes retained 98.8% of their chemical load by weight after 28 days; additional samples similarly exposed after seven freeze-thaw cycles and samples stored in sealed bottles at 5° C retained 99.1% of their initial chemical load after 28 days. Comparison of impurity profiles indicated that no impurities or significant changes in the impurity profile were introduced by microencapsulation. Results of the 4- and 20-month shelf life studies indicated that microcapsules retained greater than 98% of their chemical load when stored in sealed containers at room temperature for 4 months and greater than 99% when stored at 5° C for 20 months.

The study laboratories confirmed the identity of the microcapsules with infrared spectroscopy and analyzed the chemical load of the microcapsules using GC by systems similar to system F. GC analyses indicated a chemical load of 53.2% ± 0.8% at the beginning of the 15-day studies and 52.4% at the beginning of the 14-week studies. To ensure stability, the microcapsules were stored at room temperature, protected from light, during the 15-day studies and at approximately 5° C, protected from light and moisture, during the 14-week studies. The study laboratories monitored the stability of the microencapsulated chemical during the studies with GC by system F (15-day studies) or I (14-week studies); no loss of 1,1,2,2-tetrachloroethane was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once during the 15-day studies and at least every 3 weeks during the 14-week studies by mixing microencapsulated 1,1,2,2-tetrachloroethane with feed (Table F2). In the 15-day studies, placebo and/or loaded microcapsules were combined with feed to a concentration of 10% microcapsules; in the 14-week studies, the concentrations of microcapsules in feed were 0.86% for rats and 1.7% for mice. A premix was prepared by hand and then blended with additional feed in a twin-shell blender for 15 minutes. The dose formulations were kneaded and mixed manually and then mixed for an additional 15 minutes in the blender. In the 15-day studies, dose formulations were stored in plastic bags, protected from light, at room temperature for up to 3 weeks; dose formulations for the 14-week studies were stored in plastic bags, protected from light and moisture, at 5° C for up to 4 weeks.

Homogeneity and stability studies of a dose formulation containing 0.5% microencapsulated 1,1,2,2-tetrachloroethane were performed using GC system J. Homogeneity was confirmed, and stability studies indicated that samples were stable for 33 days when stored at 5° C. Samples stored at room temperature for 4 days, open to air and light, or for 33 days, protected from air and light, had small but significant losses of 1,1,2,2-tetrachloroethane.

The study laboratories performed homogeneity studies of the 3,325 and 53,200 ppm dose formulations for the 15-day studies and the 268, 589, 4,600, and 9,100 ppm dose formulations for the 14-week studies, as well as stability studies of the 268 ppm dose formulation, using GC by a system similar to system F (15-day studies) or by system I (14-week studies). Homogeneity was confirmed, and stability was confirmed for 28 days for dose formulations stored at room temperature or at approximately 5° C and for 1 week for dose formulations stored at room temperature under simulated animal room conditions, open to air and light.

Periodic analyses of the dose formulations were conducted by the study laboratories using GC by a system similar to system F (15-day studies) or by system I (14-week studies). During the 14-week studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples of these dose formulations were also analyzed (Table F3). Of the dose formulations analyzed, 13 of 15 for rats and 12 of 15 for mice were within 10% of the target concentrations, with no value greater than 111% of the target concentration. Five dose formulations with concentrations that were only slightly outside the 10% criterion were considered suitable for use in the studies. For the animal room samples, 12 of 15 for rats and 8 of 15 for mice were within 10% of the target

concentrations; these results were attributed to environmental degradation of the microcapsule matrix, ability of the animals to separate feed from microcapsules, and/or analytical variation.

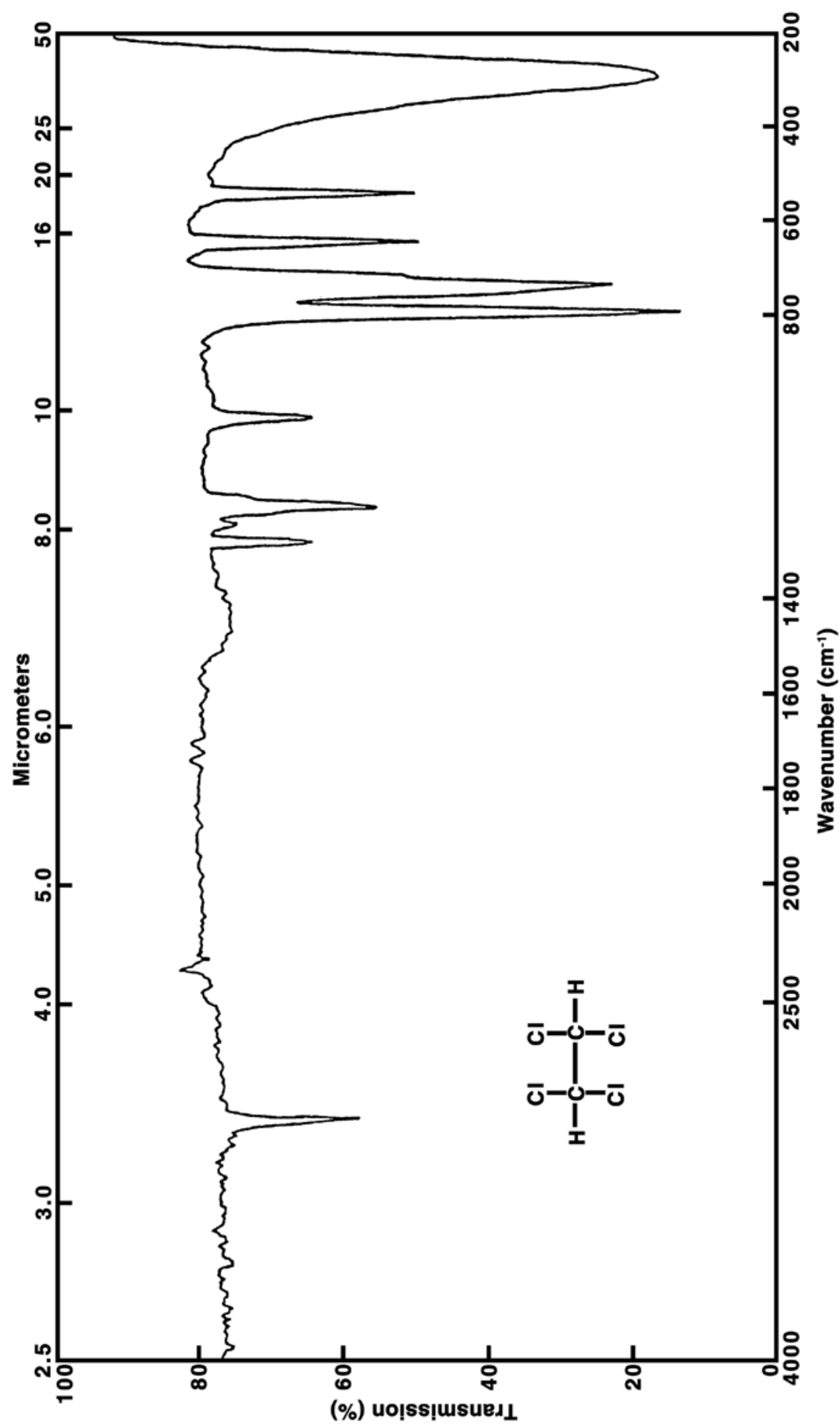

Figure F1
Infrared Absorption Spectrum of 1,1,2,2-Tetrachloroethane

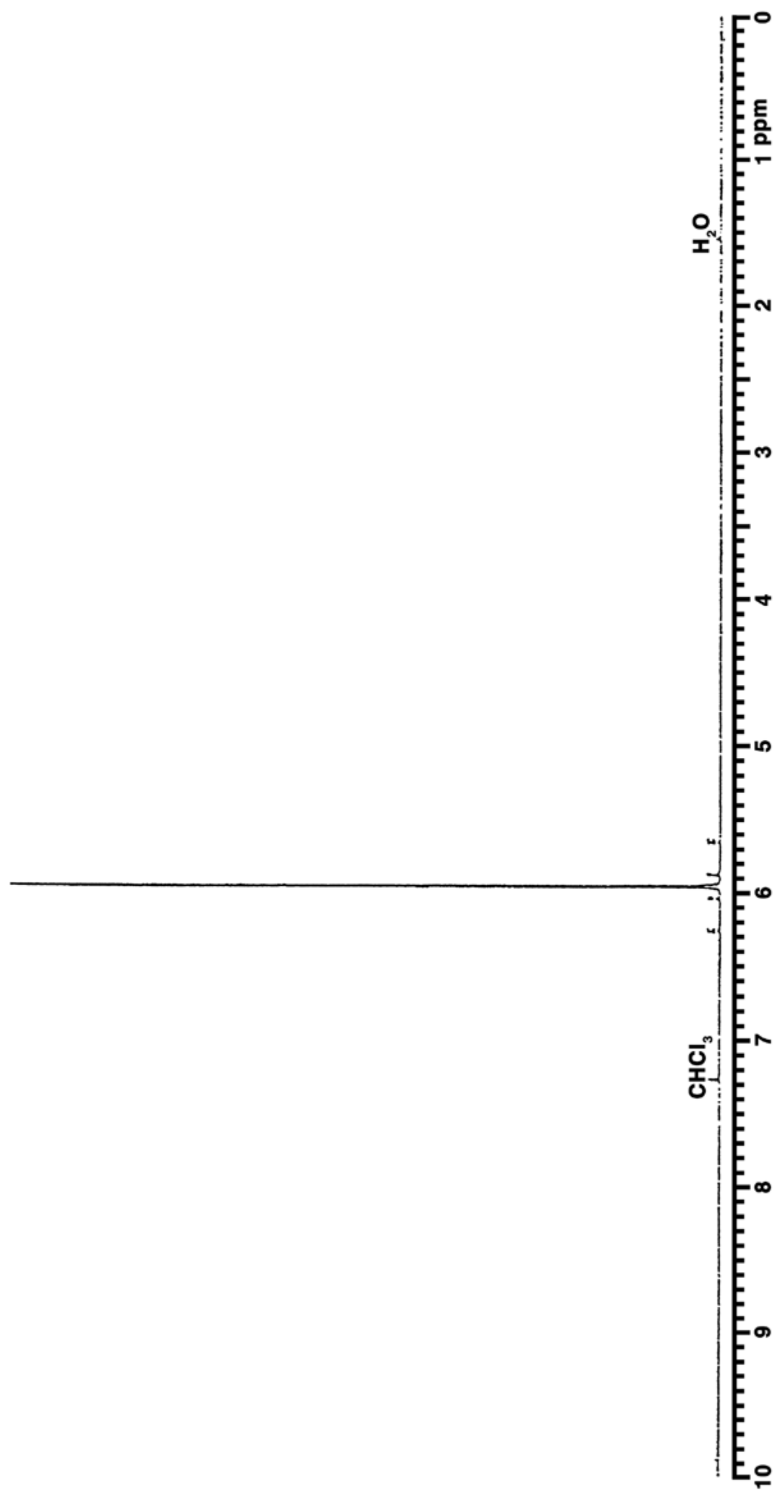

Figure F2
Nuclear Magnetic Resonance Spectrum of 1,1,2,2-Tetrachloroethane

TABLE F1
Gas Chromatography Systems Used in the Feed Studies of 1,1,2,2-Tetrachloroethane[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A Flame ionization	80/100 mesh Carbopack C/ 0.1% SP-1000, 1.8 m x 4 mm, (Supelco, Bellefonte, PA)	Nitrogen at 70 mL/minute	Isothermal at 165° C or 50° C for 2.5 or 5 minutes, then 10° C/minute to 220° C
System B Flame ionization	DB-5 Megabore, 30 m × 0.53 mm, (J&W Scientific, Folsom, CA)	Helium at 7 mL/minute	Isothermal at 60° C or 30° C for 5 minutes, then 10° C/minute to 250° C
System C Mass spectrometry with full mass scan (70 eV; scan rate 1.00 seconds; multiplier voltage –1,750 or –1,800 V)	Megabore DB-624, 30 m × 0.53 mm, 3.0-µm film (J&W Scientific)	Helium at 10 mL/minute	30° C for 3 minutes, then 8° C/minute to 220° C
System D Mass spectrometry with selected ion monitoring (70 eV; scan rate 0.900 seconds and multiplier voltage –2,200 V for identification; scan rate 0.700 seconds and multiplier voltage –2,000 V for quantitation)	Megabore DB-624, 30 m × 0.53 mm, 3.0-µm film (J&W Scientific)	Helium at 10 mL/minute	30° C for 3 minutes, then 8° C/minute to 220° C
System E Flame ionization	1% SP-1000 on 60/80 mesh Carbopak B, 1.8 m × 4 mm, (Supelco)	Nitrogen at 70 mL/minute	50° C for 5 minutes, then 10° C/minute to 250° C
System F Flame ionization	20% SP-2100/0.1% Carbowax 1500 on 100/120 mesh Supelcoport, 1.8 m × 2 mm, (Supelco)	Nitrogen at 30 mL/minute	70° C to 120° C at 10° C/minute, held 10 minutes
System G Electron capture	20% SP-2100/0.1% Carbowax 1500 on 100/120 mesh Supelcoport, 1.8 m × 2 mm, (Supelco)	Nitrogen at 30 mL/minute	100° C for 6 minutes, then 10° C/minute to 170° C

TABLE F1
Gas Chromatography Systems Used in the Feed Studies of 1,1,2,2-Tetrachloroethane

Detection System	Column	Carrier Gas	Oven Temperature Program
System H			
Flame ionization	80/100 mesh Carbopak C/ 0.1% SP-1000, 1.8 m × 4 mm, (Supelco)	Nitrogen at 70 mL/minute	50° C for 5 minutes, then 10° C/minute to 220° C, held 20 minutes
System I			
Electron capture	J&W DB-1 Megabore, 30 m × 0.53 mm, (J&W Scientific)	Nitrogen at approximately 9 mL/minute	Isothermal at 135° C
System J			
Flame ionization	20% SP-2100/0.1% Carbowax 1500 on 100/120 mesh Supelcoport, 1.8 m × 2 mm, (Supelco)	Nitrogen at 30 mL/minute	Isothermal at 75° C

[a] Gas chromatographs were manufactured by Varian, Inc. (Palo Alto, CA) (systems A, B, E-H, and J), Perkin Elmer (Norwalk, CT) (systems C and D), and Hewlett-Packard (Palo Alto, CA) (system I).

TABLE F2
Preparation and Storage of Dose Formulations in the Feed Studies of 1,1,2,2-Tetrachloroethane

15-Day Studies	14-Week Studies
Preparation	
A premix of microencapsulated 1,1,2,2-tetrachloroethane and/or placebo microcapsules and feed was prepared by hand and then layered with additional feed into a twin-shell blender and blended for 15 minutes. The dose formulations were removed from the blender, kneaded manually, and then returned to the blender and mixed for an additional 15 minutes. Dose formulations were prepared once.	Same as the 15-day studies; the dose formulations were prepared at least every 3 weeks.
Chemical Lot Number	
B17	B17
Maximum Storage Time	
3 weeks	4 weeks
Storage Conditions	
Stored in plastic bags, protected from light, at room temperature	Stored in plastic bags, protected from light and moisture, at 5° C
Study Laboratory	
TSI Mason Laboratories (Worcester, MA)	Microbiological Associates, Inc. (Bethesda, MD)

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of 1,1,2,2-Tetrachloroethane

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
May 24, 1993	May 25, 1993[b]	268	293	+9
		589	656	+11
		1,180	1,450	+23
		2,300	2,440	+6
		4,600	5,350	+16
	May 28, 1993[c]	268	248	−7
		589	589[d]	0
		1,180	1,100	−7
		2,300	2,300	0
		4,600	5,100	+11
	June 16, 1993[e]	268	322[b]	+20
		589	754[b]	+28
		1,180	1,380[b]	+17
		2,300	2,420[f]	+5
		4,600	5,070	+10
	June 16 and July 7, 1993[e]	268	254[c]	−5
		589	528[c]	−10
		1,180	1,030[c]	−13
		2,300	2,360[g]	+3
July 13, 1993	July 14, 1993	268	263[f]	−2
		589	652	+11
		1,180	1,450[b]	+23
		2,300	2,210	−4
		4,600	4,630	+1
	July 14-15, 1993	268	265[g]	−1
		1,180	1,200[c]	+2
	August 16, 1993[e]	268	253	−6
		589	575	−2
		1,180	1,140	−3
		2,300	2,180	−5
		4,600	4,480	−3

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of 1,1,2,2-Tetrachloroethane

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
August 12, 1993	August 13, 1993	268	298[f]	+11
		589	616	+5
		1,180	1,100	−7
		2,300	2,410[f]	+5
		4,600	5,160[b]	+12
	August 13 and 16, 1993	268	287[g]	+7
		2,300	2,350[g]	+2
		4,600	4,540[c]	−1
	October 1, 1993[e]	268	276	+3
		589	645	+10
		1,180	1,310	+11
		2,300	2,530	+10
		4,600	5,480	+19
Mice				
May 21, 1993	May 25, 1993[b]	589	650	+10
		1,120	1,240	+11
		2,300	2,330	+1
		4,550	5,580	+23
		9,100	11,300	+24
	May 28, 1993[c]	589	607	+3
		1,120	977	−13
		2,300	2,290	0
		4,550	5,040	+11
		9,100	10,100	+11
	June 16, 1993[e]	589	741[b]	+26
		1,120	1,360[b]	+21
		2,300	2,520	+10
		4,550	4,800	+5
		9,100	10,600	+16
	July 7, 1993[e]	589	499[c]	−15
		1,120	869[c]	−22

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of 1,1,2,2-Tetrachloroethane

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
July 2, 1993	July 7, 1993	589	616[f]	+5
		1,120	1,150[f]	+3
		2,300	2,370	+3
		4,550	4,790	+5
		9,100	9,730	+7
	July 7-8, 1993	589	601[g]	+2
		1,120	1,160[g]	+4
	July 30, 1993[e]	589	491	−17
		1,120	913	−18
		2,300	2,270[f]	−1
		4,550	4,810	+6
		9,100	9,520	+5
	July 30 and August 13, 1993[e]	2,300	2,430[g]	+6
August 13, 1993	August 13, 1993	589	680[f]	+15
		1,120	1,150	+3
		2,300	2,470	+7
		4,550	4,920	+8
		9,100	9,680	+6
	August 13 and 16, 1993	589	593[g]	+1
	October 1, 1993[e]	589	661	+12
		1,120	1,190	+6
		2,300	2,350	+2
		4,550	5,220	+15
		9,100	9,850	+8

[a] Results of duplicate analyses
[b] Reanalyzed due to higher-than-expected results
[c] Results of reanalysis
[d] Results of single analysis
[e] Animal room samples
[f] Duplicate analyses indicated S/L (smallest/largest) ratio was less than 0.90; therefore, a third aliquot of the dose formulation was analyzed.
[g] Results of triplicate analyses

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Chemical	TOX No.
Hexachloro-1,3-butadiene	1
n-Hexane	2
Acetone	3
1,2-Dichloroethane	4
Cobalt Sulfate Heptahydrate	5
Pentachlorobenzene	6
1,2,4,5-Tetrachlorobenzene	7
D & C Yellow No. 11	8
o-Cresol, *m*-Cresol, and *p*-Cresol	9
Ethylbenzene	10
Antimony Potassium Tartrate	11
Castor Oil	12
Trinitrofluorenone	13
p-Chloro-α,α,α-trifluorotoluene	14
t-Butyl Perbenzoate	15
Glyphosate	16
Black Newsprint Ink	17
Methyl Ethyl Ketone Peroxide	18
Formic Acid	19
Diethanolamine	20
2-Hydroxy-4-methoxybenzophenone	21
N, N-Dimethylformamide	22
o-Nitrotoluene, *m*-Nitrotoluene, and *p*-Nitrotoluene	23
1,6-Hexanediamine	24
Glutaraldehyde	25
Ethylene Glycol Ethers	26
Riddelliine	27
Tetrachlorophthalic Anhydride	28
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Dibutyl Phthalate	30
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