National Toxicology Program
Toxicity Report Series
Number 55

NTP Technical Report on the Toxicity Studies of

trans-1,2-Dichloroethylene

(CAS No. 156-60-5)

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

April 2002

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

FOREWORD

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

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

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

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

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Toxicity Study Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Information Service (EHIS) http://ehis.niehs.nih.gov (800-315-3010 or 919-541-3841). In addition, printed copies of these reports are available from EHIS as supplies last.

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Nancy B. Ress, Ph.D., Study Scientist

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

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CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

N.B. Ress, Ph.D., Study Scientist
J.R. Bucher, Ph.D.
R.S. Chhabra, Ph.D.
J. Mahler, D.V.M.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
M.K. Vallant, B.S., M.T.
K.L. Witt, M.S., ILS, Inc.

Microbiological Associates, Inc.
Conducted studies and evaluated pathology findings

M.L. Wenk, Ph.D., Principal Investigator
A. Allen, D.V.M., Ph.D.
R.M. Kovatch, D.V.M.
J. Miller, D.V.M.
J.M. Pletcher, M.P.H., D.V.M.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator

NTP Pathology Review
Evaluated slides and prepared pathology report (16 August 1996)

J.C. Seely, D.V.M., Chairperson
PATHCO, Inc.
J. Mahler, D.V.M.
National Toxicology Program

Environmental Health Research and Testing, Inc.
Provided sperm motility and vaginal cytology evaluations

T. Cocanougher, B.A.
D.K. Gulati, Ph.D.
S. Russell, B.A.

Analytical Sciences, Inc.
Provided statistical analyses

P.W. Crockett, Ph.D., Principal Investigator
L.J. Betz, M.S.
K.P. McGowan, M.B.A.
J.T. Scott, M.S.

Biotechnical Services, Inc.
Prepared Toxicity Study Report

S.R. Gunnels, M.A., Principal Investigator
D.C. Serbus, Ph.D.
W.D. Sharp, B.A., B.S.

PEER REVIEW

The draft report on the toxicity studies of *trans*-1,2-dichloroethylene 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.

Michelle Medinsky, Ph.D.
Durham, NC

Douglas Wolfe, Ph.D.
U.S. Environmental Protection Agency
Research Triangle Park, NC

SUMMARY

Background: 1,2-Dichloroethylene is used as a solvent for waxes and resins, in the extraction of rubber, as a refrigerant, and in the manufacture of artificial pearls. More than 1 million pounds are used annually in the United States. The chemical exists in two isomeric states, *cis* and *trans*; the *trans* isomer is used more widely in industry, and thus we tested that form.

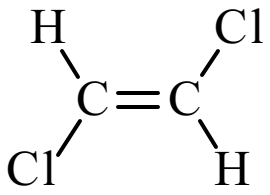
Methods: Because 1,2-dichloroethylene is both volatile and insoluble in water, we prepared starch microcapsules containing the chemical and mixed them into the feed of rats and mice to test the toxicity of the chemical. For every dose group, 5% of the feed mixture consisted of starch microcapsules; the doses of *trans*-1,2-dichloroethylene ranged from 1,470 to 23,500 ppm. The highest doses were approximately 3,200 mg/kg body weight for rats and approximately 8,000 mg/kg for mice.

Results: Male rats and male and female mice receiving the highest concentrations of *trans*-1,2-dichloroethylene weighed less than the control animals. Liver weights of exposed female rats were greater than those of the control animals, and kidney weights of exposed rats were decreased. However, no lesions associated with the chemical were observed in the exposed rats or mice. *trans*-1,2-Dichloroethylene was not mutagenic in any of the genetic toxicity tests performed.

Conclusion: Very little toxicity was associated with ingestion of microencapsulated *trans*-1,2-dichloroethylene by rats or mice. The animals could have tolerated even higher doses than those used in this study with no adverse effects.

CONTENTS

ABSTRACT



***trans*-1,2-DICHLOROETHYLENE**

CAS Number: 156-60-5

Chemical Formula: $C_2H_2Cl_2$ Molecular Weight: 96.95

Synonyms: *trans*-Acetylene dichloride; (E)-1,2-dichloroethene; (E)-(9Cl)-1,2-dichloroethene; *trans*-1,2-dichloroethene; (E)-1,2-dichloroethylene; *trans*-dichloroethylene

1,2-Dichloroethylene exists in two isomeric states: *trans*-1,2-dichloroethylene and *cis*-1,2-dichloroethylene. The *trans* isomer is used more widely in industry than the *cis* isomer. *trans*-1,2-Dichloroethylene is used as a solvent for waxes, resins, and acetylcellulose. It is also used in the extraction of rubber, as a refrigerant, and in the manufacture of pharmaceuticals and artificial pearls. F344/N rats and B6C3F$_1$ mice were administered *trans*-1,2-dichloroethylene in microcapsules in feed for 14 weeks. Animals were evaluated for clinical pathology, reproductive system effects, and histopathology. Genetic toxicity studies were conducted *in vitro* in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells, and *in vivo* in mouse bone marrow cells and peripheral blood erythrocytes.

In the 14-week feed studies, groups of 10 male and 10 female rats and mice were fed diets containing microcapsules with a chemical load of 45% *trans*-1,2-dichloroethylene. Dietary concentrations of 3,125, 6,250, 12,500, 25,000, and 50,000 ppm microencapsulated *trans*-1,2-dichloroethylene resulted in average daily doses of 190, 380, 770, 1,540, and 3,210 mg/kg for male rats; 190, 395, 780, 1,580, and 3,245 mg/kg for female rats; 480, 920, 1,900, 3,850, and 8,065 mg/kg for male mice; and 450, 915, 1,830, 3,760, and 7,925 mg/kg for female mice. Additional groups of 10 male and 10 female rats and mice served as untreated and vehicle controls. There were no exposure-related deaths of rats or mice. Mean body weights of male rats and male and female mice in the 50,000 ppm groups were

significantly less than those of the vehicle controls. The mean body weight gains of female mice in the 12,500 and 25,000 ppm groups were also significantly less than that of the vehicle controls.

On day 21 and at week 14, there were mild decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts in groups of male and female rats in the 25,000 and 50,000 ppm groups. At week 14, these effects were seen in male rats exposed to 6,250 and 12,500 ppm. There were no exposure-related alterations in clinical chemistry parameters in rats or mice.

The liver weights of female rats exposed to 6,250 ppm or greater were significantly greater than those of the vehicle controls. The absolute kidney weights of male rats exposed to 25,000 or 50,000 ppm were significantly decreased. No gross or microscopic lesions were observed in rats or mice that could be attributed to *trans*-1,2-dichloroethylene exposure.

Neither *cis*-, *trans*-, nor *cis,trans*-1,2-dichloroethylene was mutagenic in *S. typhimurium* strain TA97 (*cis* isomer only), TA98, TA100, TA1535, or TA1537, with or without S9 metabolic activation enzymes. In CHO cells *in vitro*, *cis*-1,2-dichloroethylene induced sister chromatid exchanges (SCEs) in the absence of S9; with S9, the single trial that was performed yielded equivocal results. The *cis,trans* isomer induced significant increases in SCEs in cultured CHO cells with and without S9. In contrast to these positive results, *trans*-1,2-dichloroethylene gave negative results in the SCE test, with and without S9. Neither *cis*-, *trans*-, nor *cis,trans*-1,2-dichloroethylene induced chromosomal aberrations (Abs) in cultured CHO cells, with or without S9. *In vivo*, no induction of SCEs or Abs was noted in bone marrow cells of male mice administered *cis*- or *trans*-1,2-dichloroethylene by intraperitoneal injection once, with sampling performed 23 hours (for SCE analyses) or 17 hours (for Abs analyses) after injection. In addition, negative results were obtained in a peripheral blood micronucleus test in male and female mice administered *trans*-1,2-dichloroethylene in microcapsules in feed for 14 weeks.

Very little toxicity was associated with ingestion of microencapsulated *trans*-1-2-dichloroethylene. Histopathology and clinical chemistry data, combined with body and organ weight data, revealed that the maximum tolerated dose was not reached in these studies.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

The chemical and physical properties of *cis*-, *trans*-, and *cis,trans*-1,2-dichloroethylene are listed in Table 1.

TABLE 1
Chemical and Physical Properties of *cis*-, *trans*-, and *cis,trans*-1,2-Dichloroethylene[a]

Parameter	Isomer		
	cis	***trans***	***cis,trans* Mixture**
Physical state	Liquid	Liquid	Liquid (60% *cis*, 40% *trans*)
Color	Colorless	Colorless	Colorless
Odor	Sweetish	Sweetish	Ethereal, slightly acrid
Melting point	−80.5° C	−50° C	−50° C
Boiling point	60.3° C at 760 mm Hg	48.35° C at 760 mm Hg	Not reported
Specific gravity	1.2743 (25/4° C)	1.2489 (25/4° C)	1.27 (25/4° C)
Solubility	0.35 g per 100 mL water at 20° C; soluble in alcohol, ether, benzene, chloroform, acetone, other organic solvents	0.63 g per 100 mL water at 20° C; soluble in alcohol, ether, benzene, chloroform, acetone, other organic solvents	Soluble in alcohol, ether, benzene, chloroform, acetone, other organic solvents
Log octanol/water partition coefficient	1.86	2.06	Not reported
Threshold limit value	200 ppm	200 ppm	200 ppm

[a] Threshold limit values were taken from the American Conference of Governmental Industrial Hygienists (2000); all other data were taken from the Hazardous Substances Data Bank (2000).

PRODUCTION, USE, AND HUMAN EXPOSURE

1,2-Dichloroethylene is synthesized by direct chlorination of acetylene at 40° C, by dehydrochlorination of 1,1,2-trichloroethane at 500° C, and by reduction of 1,1,2,2-tetrachloroethane with steam over an iron catalyst. The *trans* isomer is separated from the mixture through fractional distillation (HSDB, 2000).

trans-1,2-Dichloroethylene is listed as a high-production-volume chemical by the U.S. Environmental Protection Agency (USEPA, 2001) because more than 1 million pounds are produced annually. In 1977, one United States company produced 10 to 50 million pounds and another produced 1 to 10 million pounds of *cis,trans*-1,2-dichloroethylene. For the mixed isomers, one company reported production to be less than 1,000 pounds, and another estimated production of 0.1 to 1 million pounds. No production estimates for the *trans* isomer were reported. The only manufacturer of the *cis* isomer reported production of 0.1 to 10 million pounds (ATSDR, 1990).

Industrial use of the 1,2-dichloroethylenes is not widespread because of their high flammability. However, the isomeric mixture is used as a chemical intermediate for the synthesis of chlorinated solvents. It also is used as a solvent for waxes, resins, camphor, and acetylcellulose and as an extractant for rubber and for oil and fat from fish. *cis,trans*-1,2-Dichloroethylene is used as a refrigerant and in the manufacture of pharmaceuticals and artificial pearls (HSDB, 2000). The *trans* isomer has more industrial uses than either the *cis* isomer or the mixture, and it has been identified in cleaning agents such as Freon MCA and Freon SMT (Hurtt *et al.*, 1993; HSDB, 2000). *cis*-1,2-Dichloroethylene is used in the manufacture of vulcanized rubber and as a retarding agent in fermentation (HSDB, 2000).

Nonoccupational human exposure to *cis*-, *trans*-, and *cis,trans*-1,2-dichloroethylene may occur through ingestion of contaminated drinking water and/or inhalation of contaminated air. More than 4 million people are estimated to receive drinking water contaminated with these isomers (USEPA, 1984a). Current drinking water standards are 70 µg/L for *cis*-1,2-dichloroethylene and 100 µg/L for *trans*-1,2-dichloroethylene (Pontius, 1999). 1,2-Dichloroethylene has been detected in groundwater at concentrations up to 3,900 µg/L and in drinking water at concentrations ranging from 2 to 120 µg/L (ATSDR, 1990).

In 1997, releases of 17 pounds of 1,2-dichloroethylene in water and 7,757 pounds in air were reported; no releases on land were reported (USEPA, 1999a). Both *cis*- and *trans*-1,2-dichloroethylene have been identified at concentrations of 1 to 5 µg per dry standard cubic meter in emissions from hazardous waste combustion (Trenholm, 1998). Additionally, 1,2-dichloroethylene is present at 204 National Priority List (NPL) sites. It has been detected in the groundwater at 183 NPL sites, in the air at four NPL sites, and in the surface water at 18 NPL sites (USEPA, 1999b). When released in the soil, 1,2-dichloroethylene evaporates or leaches into the groundwater, where it is relatively stable. 1,2-Dichloroethylene is long lived in the atmosphere but may be lost through reaction with hydroxyl radicals or scavenged by rain (HSDB, 2000). Thus, there is a potential for human exposure through ingestion of contaminated water or inhalation of contaminated air.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

1,2-Dichloroethylenes bind to the active site of CYP450, resulting in inhibition of their own metabolism (Costa and Ivanetich, 1982). Both *cis*- and *trans*-1,2-dichloroethylene are metabolized by CYP2E1 to an epoxide intermediate that has been shown to covalently bind to proteins, forming *S*-methylcysteine amino acid adducts (Maiorino *et al.*, 1982). The epoxide intermediate nonenzymatically rearranges to form 2,2-dichloroacetaldehyde (Costa and Ivanetich, 1982), which is enzymatically converted to 2,2-dichloroethanol or 2,2-dichloroacetate via aldehyde or alcohol dehydrogenase (Bonse *et al.*, 1975; Costa and Ivanetich, 1982). These metabolites have been identified in an experiment performed to determine the uptake and metabolism of *cis*- and *trans*-1,2-dichloroethylene (Bonse *et al.*, 1975). In this study, the livers of female Wistar rats were perfused at a constant rate with *cis*- or *trans*-1,2-dichloroethylene. After 60 minutes, the uptake of the *trans* isomer was slower than that of the *cis* isomer, suggesting that the metabolism of the *cis* isomer is more rapid than that of the *trans* isomer. These results are supported by the work of Costa and Ivanetich (1982), who showed that the *cis* isomer is metabolized more readily than the *trans* isomer.

trans-1,2-Dichloroethylene has been shown to selectively inhibit CYP2E1 both *in vitro* and *in vivo*. In a study by Mathews *et al.* (1998), groups of three male Fischer 344 rats were administered intraperitoneal injections of 25, 50, 100, 400, or 800 mg/kg *trans*-1,2-dichloroethylene. Maximal CYP2E1 inhibition occurred 2 hours after administration of 100 mg/kg and extended to 12 hours after treatment; CYP2E1 activity returned to control values within 24 hours. Total CYP450 and the activities of specific isozymes other than CYP2E1 were not significantly affected *in vivo*. However, inhibition of CYP2E1 (80%), CYP1A2, and CYP2C6 was observed *in vitro* (Mathews *et al.*, 1998).

Further kinetic characterization of CYP2E1 inhibition by *cis*- and *trans*-1,2-dichloroethylene was determined both *in vivo* and *in vitro*, and mechanisms of enzyme inhibition were evaluated with physiological models (Lilly *et al.*, 1998). Male Fischer 344 rats were pretreated with acetone (50% solution in 10% Emulphor by gavage) for 3 days to enhance CYP2E1 activity. Twenty hours after the last treatment, liver microsomes were isolated and then incubated with 62.5 to 10,000 ppm *cis*-1,2-dichloroethylene or 5 to 1,000 ppm *trans*-1,2-dichloroethylene. Following incubations, inhibition of *p*-nitrophenyl hydroxylase, a marker for CYP2E1 activity, was assessed. CYP2E1 activity was 30% of controls following incubation with 125 ppm *trans*-1,2-dichloroethylene; inhibition was maximal (approximately 19% of controls) in microsomes incubated with 500 ppm *trans*-1,2-dichloroethylene. Higher concentrations of *cis*-1,2-dichloroethylene (5,000 ppm) were required for comparable inhibition of CYP2E1 activity. Several mechanistic models were examined; however, the model that

best fit the data incorporated destruction of the enzyme-substrate complex via reaction with a reactive metabolite of *cis*- or *trans*-1,2-dichloroethylene. In a separate experiment, rats were similarly pretreated with acetone but were then exposed by inhalation to concentrations of 75 to 1,000 ppm *cis*-1,2-dichloroethylene or 5 to 25 ppm *trans*-1,2-dichloroethylene. Samples of the chamber air were taken every 10 minutes for 3 to 6 hours. Gas uptake results of *cis*- or *trans*-1,2-dichloroethylene showed lower chamber concentrations with decreasing exposure concentrations, indicating inhibition of metabolism at the higher concentrations. *In vivo* modeling experiments indicated that the mechanism of CYP2E1 inhibition by *cis*- or *trans*-1,2-dichloroethylene occurred through reaction of the activating enzyme with reactive metabolites formed during the metabolism of the dichloroethylenes.

Inhalation pharmacokinetics were studied in male Wistar rats exposed to *cis*- or *trans*-1,2-dichloroethylene using a closed inhalation chamber. In this study, a nonphysiologically constrained, two-compartment model was used to analyze the data (Filser and Bolt, 1979). For the *trans* and *cis* isomers, the zero-order V_{max} elimination rates were reported to be 0.67 and 2.4 mg/hour•kg, respectively. The authors suggested that the low maximal velocities of *trans*- and *cis*-1,2-dichloroethylene were due to the inactivation of CYP450 by reactive epoxide intermediates. In a study to compensate for enzyme inhibition-resynthesis, V_{maxC} values of 3 and 2.49 mg/hour•kg were reported for *cis*- and *trans*-1,2-dichloroethylene, respectively (Gargas *et al.,* 1990). In a more recent study by Lilly *et al.* (1998), several physiologically based pharmacokinetic models were tested based on both *in vitro* and *in vivo* experiments (as described previously). Assuming interaction between the reactive metabolites and the enzyme-substrate complex, it was determined that the *in vivo* model was the most biologically relevant upon comparison with V_{maxC} values for other structurally similar compounds. Using this model, V_{maxC} values were determined to be 4.53 mg/hour•kg for the *cis* isomer and 4.27 mg/hour•kg for the *trans* isomer.

Humans

No information on the distribution, metabolism, or excretion of *cis*-, *trans*-, or *cis,trans*-1,2-dichloroethylene in humans was found in the literature. However, it has been reported that 72% to 75% of inhaled *trans*-1,2-dichloroethylene is absorbed in the lungs (ATSDR, 1990). The 1,2-dichloroethylenes are likely absorbed readily through inhalation, ingestion, and dermal exposure due to their high lipid solubilities and low molecular weight (USEPA, 1984b).

TOXICITY

Experimental Animals

The LD_{50} and LC_{50} values for *trans*-1,2-dichloroethylene administered to rodents by various routes are provided in Table 2.

In an acute toxicity study, groups of four to six adult Holtzman rats were administered either *cis*- or *trans*-1,2-dichloroethylene in corn oil by gavage at 400 or 1,500 mg/kg (Jenkins *et al.*, 1972). In the 1,500 mg/kg groups, the *cis* isomer elicited a greater biochemical response than the *trans* isomer, although all effects were minimal. Administration of 400 or 1,500 mg/kg *cis*-1,2-dichloroethylene caused a significant increase in liver alkaline phosphatase activity. In animals exposed to 1,500 mg/kg, liver glucose-6-phosphatase, liver tyrosine transaminase, and plasma alanine aminotransferase activities were significantly increased. Rats exposed to 400 mg/kg of the *trans* isomer showed increased liver glucose-6-phosphatase activity, while tyrosine transaminase activity was increased in the 1,500 mg/kg group.

The acute toxicity of *trans*-1,2-dichloroethylene also was determined in male and female CD-1 mice (Barnes *et al.,* 1985). For 14 days, groups of 9 or 10 mice were administered daily gavage doses of 21 or 210 mg/kg *trans*-1,2-dichloroethylene in a 1:9 solution of Emulphor and deionized water. There were no significant changes

TABLE 2
LD_{50} and LC_{50} Values for *trans*-1,2-Dichloroethylene

Route	Species, Strain, Gender	LD_{50}/LC_{50}	Reference
Gavage	Rats		
	Wistar, female	1,275 mg/kg[a]	Freundt *et al.*, 1977
	Sprague-Dawley, male	7,900 mg/kg	Hayes *et al.*, 1987
	Sprague-Dawley, female	9,900 mg/kg	Hayes *et al.*, 1987
	Mice		
	CD-1, male	2,221 mg/kg	Munson *et al.*, 1982
	CD-1, female	2,391 mg/kg	Munson *et al.*, 1982
Intraperitoneal injection	Rats		
	Wistar, female	7,650 mg/kg	Freundt *et al.*, 1977
	Mice		
	NMR, female	4,022 mg/kg	Freundt *et al.,* 1977
Inhalation	Mice		
	Unspecified	22,000 mg/m^3	ATSDR, 1990

[a] Possible aspiration of dose, as evidenced by pulmonary damage

in organ or body weights in any dosed group. However, slight decreases in fibrinogen concentrations (12%), prothrombin times, and lactate dehydrogenase activities were observed in rats administered 210 mg/kg per day.

In another study to assess the subacute effects of oral exposure to the *cis* isomer, groups of 10 male and 10 female Sprague-Dawley rats were administered 32, 98, 293, or 878 mg/kg in corn oil by gavage daily for 14 days (McCauley *et al.*, 1995). A complete necropsy and hematology and clinical chemistry evaluations were performed on each animal. Male and female rats administered 878 mg/kg per day were lethargic and had secretions around the nose and mouth. In addition, a 10% decrease in body weight occurred in males in this group, while no body weight effects were observed in females. Dose-related increases in liver weights were observed in males and females in all dosed groups. Kidney weights of females in the 293 and 878 mg/kg groups and testis weights of males in the 878 mg/kg group were increased.

The short-term toxicity of the *trans* isomer also was determined in an inhalation study by Freundt *et al.* (1977). Adult female SPF Wistar rats and NMRI mice were exposed to 200 ppm *trans*-1,2-dichloroethylene via inhalation 8 hours per day for 5 or 10 days or 200, 1,000 or 3,000 ppm by inhalation for one 8-hour period. The blood, lung, heart, liver, kidney, spleen, brain, muscle (quadriceps), and sciatic nerve were collected and examined. In all exposure groups, increases in fatty degeneration of hepatic lobules and Kupffer cells were observed. Additionally, a pronounced increase in the appearance of pulmonary capillary hyperemia and distention of the alveolar septum occurred and became more pronounced with increasing exposure time and concentration. Exposure to 1,000 ppm for 8 hours resulted in decreased numbers of red and white blood cells in rats. In mice, exposure to 3,000 ppm for 8 hours resulted in fibrous swelling and hyperemia of the cardiac muscle with barely maintained striation.

In a subchronic study to assess the oral toxicity of the *cis* isomer, groups of 10 male and 10 female Sprague-Dawley rats were administered 10, 32, 98, or 206 mg/kg per day in corn oil by gavage for 90 days (McCauley *et al.*, 1995). Significant decreases in liver-weight-to-body-weight ratios of males and females and increases in kidney weights of males occurred in all dosed groups. No significant histopathologic changes were seen in males or females. Serum phosphorus concentrations in dosed females and serum calcium concentrations in dosed males were slightly increased. Additionally, males in the 206 mg/kg group exhibited a decreased blood urea nitrogen concentration. Overall, *cis*-1,2-dichloroethylene exhibited some toxicity at all doses.

The *trans* isomer was evaluated for subchronic toxicity in groups of 24 male and 24 female CD-1 mice administered 0.1, 1.0, or 2.0 mg/mL in drinking water (Barnes *et al.*, 1985). Based on the amounts of water ingested, males received a daily dose of 17, 175, or 387 mg/kg; females received a daily dose of 23, 244, or 452 mg/kg. No changes in terminal body weights or gross pathology were observed, and clinical chemistry

effects were considered to be minimal. The most noteworthy effects included a 21% decrease in liver glutathione in males in the 2 mg/mL group and significant decreases in thymus weights of females in the 1 and 2 mg/mL groups.

In a 90-day study, CD rats were administered *trans*-1,2-dichloroethylene in drinking water at daily doses of 402, 1,311, or 3,114 mg/kg for males and 353, 1,257, or 2,809 mg/kg for females (Hayes *et al.*, 1987). In female rats exposed to 1,257 or 2,809 mg/kg, significant decreases in kidney weights were observed. No other chemical-related effects on body weight or on hematology or clinical chemistry parameters were found.

In a study to assess the immunotoxicity of *trans*-1,2-dichloroethylene, groups of 10 to 12 male CD-1 mice were administered 22 or 222 mg/kg by gavage (vehicle not indicated) for 14 days. Treatment did not affect body weight, organ weights (brain, kidney, liver, lungs, spleen, or thymus), lymphocyte count, hematocrit value, or hemoglobin concentration at either dose. Immunologically, the *trans* isomer produced a slight but not dose-dependent inhibition in the number of cells forming IgM antibodies to sheep erythrocytes. No change was observed in cell-mediated immunity evaluated by measuring the delayed hypersensitivity response to sheep erythrocytes (Munson *et al.*, 1982).

In another study to assess the immunotoxicity of *trans*-1,2-dichloroethylene, groups of 12 male and 12 female CD-1 mice were exposed to drinking water containing 17, 175, or 387 mg/kg (males) and 23, 224, or 452 mg/kg (females) for 90 days (Shopp *et al.,* 1985). At all doses, the spleen cells of male mice exhibited a decreased ability to produce antibodies against sheep erythrocytes, indicating a suppression in humoral immune status. In exposed females, macrophage induction was decreased, as evidenced by a decline in the ability of thioglycolate-recruited peritoneal exudate cells to phagocytize sheep erythrocytes.

Humans

Inhalation of 1,2-dichloroethylene vapors may result in central nervous system depression, nausea, weakness, tremors, and cramps (HSDB, 2000). Humans exposed by inhalation to 1,715 to 2,220 ppm for 5 minutes or 1,210 ppm for 10 minutes exhibited nausea, drowsiness, fatigue, vertigo, and increased intracranial pressure (ATSDR, 1990). Inhalation of 1,2-dichloroethylene vapors (duration and concentration not specified) has resulted in one fatality (Torkelson and Rowe, 1981).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Groups of 24 pregnant female Crl:CD Br rats were exposed to 0, 2,000, 6,000, or 12,000 ppm *trans*-1,2-dichloroethylene in air for 6 hours per day on gestation days 7 through 16 (Hurtt *et al.*, 1993). Animals were killed on gestation day 22 and assessed for toxicity. Maternal body weight loss was observed in dams exposed to 6,000 ppm on gestation days 11 through 13 and in dams exposed to 12,000 ppm throughout the study; weight loss paralleled decreased feed consumption in the 6,000 and 12,000 ppm groups. A narcotizing effect occurred from maternal exposure to 6,000 or 12,000 ppm. Alopecia, lethargy, and salivation were observed in the 12,000 ppm group. Significant increases in the numbers of early and total resorptions were reported in dams exposed to 6,000 or 12,000 ppm but not 2,000 ppm. Additionally, mean fetal body weights for dams exposed to 12,000 ppm were significantly decreased. Due to the maternal toxicity in the 6,000 and 12,000 ppm groups, these data should be evaluated with caution. No differences in incidence or type of malformation were observed in exposed fetuses, although a slight but nonsignificant increase in the occurrence of hydrocephalus was observed in the 12,000 ppm group.

Humans

No data were available on the reproductive toxicity of *cis*-, *trans*-, or *cis,trans*-1,2-dichloroethylene in humans.

CARCINOGENICITY

No information was found in a search of the literature regarding the carcinogenicity of *cis*-, *trans*-, or *cis,trans*-1,2-dichloroethylene in animals or humans.

GENETIC TOXICITY

The mutagenicity of *cis-, trans-,* and *cis,trans*-1,2-dichloroethylene has been tested in the *Salmonella typhimurium* microsome assay. Neither the *trans* isomer nor the isomeric mixture was mutagenic in a preincubation study using strains TA98, TA100, TA1535, and TA1537, with and without Aroclor 1254-induced rat or hamster liver S9 (Mortelmans *et al.*, 1986). The *cis* isomer was similarly tested, and negative results were obtained in these four strains and strain TA97 (Zeiger *et al.*, 1988). In a study that employed the standard plate incorporation method, positive results were reported with the *trans* isomer in strain TA100 with and without S9 and strains TA97 and TA98 with S9; no mutagenic activity was noted in strain TA104 with or without S9 (Strobel and Grummt, 1987).

The *cis* and *trans* isomers were incubated, in the presence and absence of mouse liver microsomes, with *Escherichia coli* K23, a bioauxotrophic strain, to test for back mutations in the gal+, arg+, and nad+ operons and forward mutations in the MTR operon (confers resistance to 5-methyl-DL-tryptophan). Neither *cis*- nor *trans*-1,2-dichloroethylene was mutagenic with or without metabolic activation (Greim *et al.*, 1975).

Both the *cis* and *trans* isomers induced mitotic recombination in *Saccharomyces cerevisiae* D7 (Bronzetti *et al.*, 1984). In addition, *cis*-1,2-Dichloroethylene induced a small but significant increase in gene mutations in *S. cerevisiae* D7 in the absence of S9 activation; neither isomer induced mitotic gene conversion (Bronzetti *et al.*, 1984). Additionally, negative results were observed in a mitotic gene conversion assay with *trans*-1,2-dichloroethylene in metabolically competent or S9-supplemented lines of *S. cerevisiae* D7. However, over the same concentration range, the *trans* isomer was active in an assay for aneuploidy induction (chromosome loss) in strain D61.M, with and without S9 activation (Koch *et al.*, 1988).

cis,trans-1,2-Dichloroethylene was tested for micronucleus induction using the cytokinesis block method and for DNA damage using single-cell gel electrophoresis (COMET assay) in human lymphocytes *in vitro* (Tafazoli and Kirsch-Volders, 1996). A small but significant increase in the frequency of micronucleated cells was observed at the highest concentration tested (20 mM), with and without S9 mix. However, no clear or reproducible dose-response relationship was indicated. Positive results also were seen in the COMET assay, indicating induction of DNA damage in isolated lymphocytes, at concentrations of 4 to 8 mM with S9 and 6 mM without S9. Additionally, *in vitro* micronucleus studies in lymphoblastoid cell lines with an unspecified 1,2-dichloroethylene isomer showed mixed responses. Micronucleus frequencies were significantly increased in AHH-1 and h2E1 cell lines but not in MCL-5 cells with high CYP1A activity (Doherty *et al.*, 1996). The results of special staining techniques used to discern the presence or absence of kinetochores in the induced micronuclei suggested that the increased frequencies of micronuclei were due both to structural damage and to induced aneuploidy. In contrast to the positive results observed in *in vitro* micronucleus assays, earlier tests for induction of sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster lung fibroblasts were negative with both the *cis* and *trans* isomers at concentrations up to 2 mg/mL, with and without induced rat liver S9 (Sawada *et al.*, 1987).

In their published abstract, Černá and Kypěnová (1977) reported that intraperitoneal injections of *cis*-1,2-dichloroethylene, administered daily at one-sixth of the LD_{50} value for 5 days, induced chromosomal aberrations in the bone marrow cells of female ICR mice sampled 6, 24, and 48 hours after treatment; the *trans* isomer had no effect. Furthermore, the authors reported that a dose response was established for induction of chromosomal aberrations by *cis*-1,2-dichloroethylene in a follow-up study in which the isomer was administered in a series of 10 daily injections. No data or protocol details were included in this abstract, precluding

independent evaluation of the results. In contrast to the induction of chromosomal aberrations attributed to *cis*-1,2-dichloroethylene *in vivo*, male and female CD-1 mice administered intraperitoneal injections of 280 or 490 mg/kg *cis,trans*-1,2-dichloroethylene (40% or 70% of the LD_{50}, respectively) did not have increased frequencies of micronuclei in bone marrow erythrocytes 24 or 48 hours after injection (Crebelli *et al.*, 1999). These results were part of a larger, extensive study of 10 halogenated aliphatic hydrocarbons, none of which induced a micronucleus response in mouse bone marrow.

In conclusion, the limited mutagenicity data for the isomers of 1,2-dichloroethylene show mixed results. Mostly negative results were obtained in bacterial gene mutation studies, and conflicting results were seen in mammalian cell studies for chromosomal effects, with some *in vitro* tests yielding weakly positive results and *in vivo* study results suggesting that 1,2-dichloroethylene is not active in mouse bone marrow.

STUDY RATIONALE

1,2-Dichloroethylene was recommended for toxicologic characterization by the National Cancer Institute and the USEPA under the Superfund program because of possible widespread human exposure due to its presence in drinking water and at hazardous waste sites. *trans*-1,2-Dichloroethylene was microencapsulated and administered in feed to achieve higher concentrations than would be obtainable in drinking water. The *trans* isomer was selected for subchronic toxicity studies based on the results of preliminary acute toxicity studies and its prevalence in industrial applications. In these studies, the most significant effect was a 20% reduction in final mean body weight of male rats exposed to 50,000 ppm *trans*-1,2-dichloroethylene. Based on this information, the highest dose for the 14-week studies was 50,000 ppm. In the 14-week studies, F344/N rats and $B6C3F_1$ mice were exposed to 3,125, 6,250, 12,500, 25,000, or 50,000 ppm microencapsulated *trans*-1,2-dichloroethylene in the diet for 14 weeks. In addition, genetic toxicity studies of *cis-, trans-,* and *cis,trans*-1,2-dichloroethylene were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, mouse bone marrow cells, and mouse peripheral blood erythrocytes.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF *TRANS*-1,2-DICHLOROETHYLENE

trans-1,2-Dichloroethylene was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI), in one lot (MP-02224LP). Microencapsulation was performed by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the microcapsules were assigned lot number 343-1B-A. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and the study laboratory (Appendix F). Reports on analyses performed in support of the *trans*-1,2-dichloroethylene studies are on file at the National Institute of Environmental Health Sciences.

Analyses of Neat Chemical

The chemical, a clear, colorless liquid, was identified with infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. Purity was determined using elemental analyses, Karl Fischer water analysis, free acid titration, gas chromatography (GC), and GC with mass spectrometry (GC/MS).

Elemental analyses for carbon, hydrogen, and chlorine were in agreement with the theoretical values for *trans*-1,2-dichloroethylene. Karl Fischer water analysis indicated 0.010% ± 0.001% water. Free acid titration indicated 0.0007 ± 0.0002 mEq acid per gram, equivalent to 26 ± 7 ppm as hydrochloric acid. GC by one system indicated one major peak and five impurities with a combined area of 1.25% relative to the major peak area. GC by a second system indicated one major peak and three impurities with a combined relative area of 0.69%. The overall purity of lot MP-02224LP was determined to be approximately 99%. Minor impurities were identified as chlorinated compounds by GC/MS. The study laboratory confirmed that the purity was 99% or greater.

Stability information supplied by the manufacturer indicated that neat *trans*-1,2-dichloroethylene is stable when stored frozen and protected from air, moisture, and light; the chemical was stored under these conditions throughout the 14-week studies. Stability was monitored with infrared spectroscopy and GC during preliminary pilot studies conducted by TSI Mason Laboratories (Worcester, MA); no degradation of the bulk chemical was detected.

Microcapsule Formulation and Analyses

Microcapsules loaded with neat *trans*-1,2-dichloroethylene and placebos (empty microcapsules) were prepared by the analytical chemistry laboratory with a proprietary process using food-grade, modified corn starch (CAPSUL®) 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 of *trans*-1,2-dichloroethylene, the analytical chemistry laboratory tested the microencapsulated 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, 120, and PAN). The chemical loads (amount of *trans*-1,2-dichloroethylene in the starch/sugar matrix) of freshly prepared microcapsules and of microcapsules stored under a variety of conditions were determined with GC. Major peak comparisons of the neat and microencapsulated chemical and 9-month stability studies were also performed with GC.

Microscopic examination revealed no unusual characteristics. The particles were within size specifications. The chemical load was determined to be 45.0% ± 0.3%. Microcapsules stored for 28 days under animal room conditions (open to air and light), with and without freeze-thaw cycles, or in sealed bottles at 5° C retained 93.8% of their chemical load by weight. Major peak comparisons indicated that no impurities were introduced by microencapsulation. Results of the 9-month shelf-life study indicated that microcapsules retained 91% of their chemical load when stored at 5° C and 89% when stored at room temperature; microcapsules stored at 5° C for 6 months and then at room temperature, open to air and light, for 28 days were stable.

The study laboratory confirmed the identity of the microcapsules with infrared spectroscopy and analyzed the chemical load of the microcapsules with GC. GC analyses performed at the beginning of the preliminary pilot studies indicated a chemical load of approximately 43%. Prior to the 14-week studies, GC analyses of *trans*-1,2-dichloroethylene samples from one bottle of microcapsules indicated a concentration of 37.6% ± 2.4%, which was lower than the expected concentration of 45.3%; microcapsules from six other bottles were combined and analyzed with GC. Homogeneity was confirmed, and the chemical load of 47.0% was considered to be acceptable for use in the 14-week studies. The chemical load was monitored during the 14-week studies with GC; no loss of *trans*-1,2-dichloroethylene was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared at least every 2 weeks by mixing microencapsulated *trans*-1,2-dichloroethylene with feed (Table F2); placebo microcapsules were added to maintain a starch matrix concentration of 6% in the diet. Formulations were stored in doubled plastic bags, protected from light, at 5° C for up to 4 weeks. Homogeneity and stability studies of 0.5% *trans*-1,2-dichloroethylene formulations were conducted by the analytical chemistry laboratory using GC. Homogeneity was confirmed. The *trans*-1,2-dichloroethylene formulation prepared with lot 343-1A had losses of 22.0% for samples stored for 7 days in the dark and 15.5% for samples stored for 1 day at room temperature, open to air and light; dose formulations prepared with lots 343-10TA, -11TA, and -12TA (not used in the current studies) and lot 343-1B-A were stable for 4 days when stored at room temperature, open to air and light. Additional analyses performed with GC with a 0.5% dose formulation prepared with lot 343-1B-A confirmed stability for 7 days for samples stored in a rat cage, open to air and light, at up to 50% humidity.

The study laboratory conducted homogeneity studies of 0.7% and 11.5% *trans*-1,2-dichloroethylene formulations with GC. Prior to the 14-week studies, the study laboratory also performed homogeneity studies of 3,125 and 50,000 ppm dose formulations and stability studies of a 3,125 ppm dose formulation with GC. Homogeneity of all formulations was confirmed. Stability was confirmed for 28 days for dose formulations stored in doubled plastic bags at up to 5° C, the storage conditions used during the studies.

Periodic analyses of the dose formulations were conducted by the study laboratory using GC (Table F3). All dose formulations that were not within 10% of the target concentrations were reblended and reanalyzed; all dose formulations analyzed and used for dosing were within 10% of the target concentrations.

14-WEEK STUDIES

Male and female F344/N rats and B6C3F_1 mice were obtained from Taconic Farms (Germantown, NY) and were 5 weeks old on receipt. 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 rats and mice at the beginning of the studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results were negative.

The exposure concentrations selected for use in the 14-week studies were based on the results of pilot studies in which no findings of overt toxicity were observed at exposure concentrations up to 50,000 ppm *trans*-1,2-dichloroethylene (5% in feed). The substitution of greater than 5% of the feed interferes with the availability of some essential vitamins and minerals and causes nutritional imbalances (USEPA, 1980).

Groups of 10 male and 10 female rats and mice were fed diets containing 3,125, 6,250, 12,500, 25,000, or 50,000 ppm microencapsulated *trans*-1,2-dichloroethylene for 14 weeks. Additional groups of 10 male and 10 female rats and mice received untreated feed (untreated controls) or feed containing placebo microcapsules (vehicle controls) for 14 weeks. 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 weekly. Feed consumption was recorded weekly by cage. The animals were weighed initially, weekly, and at the end of the studies. A functional observation battery was performed on rats and mice in the untreated and vehicle control groups and the 12,500, 25,000, or 50,000 ppm groups during weeks 4 and 13. Details of the study design and animal maintenance are summarized in Table 3.

Additional groups of 10 male and 10 female rats received the same exposure concentrations of *trans*-1,2-dichloroethylene as the core study animals. These rats were used for clinical pathology testing only and were not necropsied.

Blood for hematology and clinical chemistry analyses was collected from clinical pathology study rats on days 5 and 21 and from core study rats and mice (clinical chemistry only) at the end of the studies. At all time points, the animals were anesthetized with a mixture of carbon dioxide and air (70:30), and blood was collected from the retroorbital sinus. Blood for hematology determinations was placed in tubes containing potassium EDTA as the anticoagulant. The automated hematocrit value; hemoglobin concentration; erythrocyte, leukocyte, and platelet counts; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined with a Serono-Baker 9000 hematology analyzer (Serono-Baker Diagnostics, Allentown, PA). Manual hematocrit determinations were made with an Adams CT2900 Microhematocrit Centrifuge (Clay Adams, Sparks, MD). Differential leukocyte counts and erythrocyte, leukocyte, and platelet morphologies were determined by light microscopy from blood smears stained on an Ames Hema-Tek Slide Stainer (Miles Laboratory, Ames Division, Elkhart, IN) using a modified Wright's stain. Reticulocytes were stained with new methylene blue (Sigma Chemical Company, St. Louis, MO) and counted microscopically. Blood for clinical chemistry analyses was placed in tubes with no anticoagulant, allowed to clot at room temperature, and centrifuged. The sera were separated, and clinical chemistry parameters were measured with a Hitachi 717 chemistry analyzer (Boehringer Mannheim, Indianapolis, IN). Reagents were obtained from the manufacturer with the exception of the reagents

for sorbitol dehydrogenase and total bile acids, which were obtained from Sigma Chemical Company. The hematology and clinical chemistry parameters measured are listed in Table 3.

At the end of the 14-week studies, samples were collected for sperm count and motility and vaginal cytology evaluations on rats and mice in the untreated control and vehicle control groups and the 12,500, 25,000, and 50,000 ppm groups. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on rats and mice in the untreated control, vehicle control, and 50,000 ppm groups. Table 3 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 3
Experimental Design and Materials and Methods in the 14-Week Feed Studies of *trans*-1,2-Dichloroethylene

Study Laboratory
Microbiological Associates, Inc. (Bethesda, MD)

Strain and Species
Rats: F344/N
Mice: B6C3F$_1$

Animal Source
Taconic Farms (Germantown, NY)

Time Held Before Studies
Rats: 14 (males) or 15 days (females)
Mice: 12 (females) or 13 days (males)

Average Age When Studies Began
7 weeks

Date of First Exposure
Rats: 13 (male) or 14 (female) May 1993
Mice: 11 (female) or 12 (male) May 1993

Duration of Exposure
14 weeks

Date of Last Exposure
Rats: 12 (male) or 13 (female) August 1993
Mice: 10 (female) or 11 (male) August 1993

Necropsy Dates
Rats: 12 (male) or 13 (female) August 1993
Mice: 10 (female) or 11 (male) August 1993

Average Age at Necropsy
20 weeks

Size of Study Groups
10 males and 10 females

Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.

Animals per Cage
Rats: 5
Mice: 1 (male) or 5 (female)

Method of Animal Identification
Tail tattoo

Diet
NIH-07 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed once or twice (female mice) per week

Water
Tap water (Bethesda municipal supply) via automatic watering system, available *ad libitum*

TABLE 3
Experimental Design and Materials and Methods in the 14-Week Feed Studies of *trans*-1,2-Dichloroethylene

Cages
Polycarbonate (Lab Products, Inc., Rochelle Park, NJ), changed once (male mice) or twice per week

Bedding
Heat-treated hardwood chips (P.J. Murphy Forest Products, Montville, NJ), changed once (male mice) or twice per week

Racks
Stainless steel (Lab Products, Inc., Rochelle Park, NJ), changed and rotated every 2 weeks

Animal Room Environment
Temperature: 72° ± 3° F
Relative humidity: 50% ± 15%
Room fluorescent light: 12 hours/day
Room air changes: at least 10/hour

Exposure Concentrations
0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm, microencapsulated in feed

Type and Frequency of Observation
Animals were observed twice daily and were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly. Feed consumption was recorded weekly by cage.

Method of Sacrifice
Carbon dioxide asphyxiation

Necropsy
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.

Clinical Pathology
Blood was collected from the retroorbital sinus of special study rats on days 5 and 21 and from core study rats and mice at the end of the studies for hematology (rats only) and clinical chemistry.
Hematology: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials
Clinical chemistry: urea nitrogen, creatinine, total protein, albumin, cholesterol, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, 5′-nucleotidase, and bile acids

Histopathology
Complete histopathology was performed on all rats and mice in the untreated control, vehicle control, and 50,000 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 only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, uterus, and Zymbal's gland.

Functional Observation Battery
Functional observation batteries were performed on core study rats and mice in the untreated and vehicle control groups and the 12,500, 25,000, and 50,000 ppm groups during weeks 4 and 13. The following parameters were evaluated: body position, activity level, coordination of movement, gait, general behavior, head flick, head searching, compulsive biting or licking, backward walking, self-mutilation, circling, convulsions, tremors, lacrimation or chromodacryorrhea, salivation, piloerection, pupillary dilation or constriction, unusual respiration, diarrhea, excessive or diminished urination, and vocalization.

Sperm Motility and Vaginal Cytology
At the end of the studies, sperm samples were collected from all male animals in the untreated and vehicle control groups and the 12,500, 25,000, and 50,000 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, left epididymis, and left testis were weighed. Vaginal samples were collected for 12 consecutive days before the end of the studies from all females in the untreated and vehicle control groups and the 12,500, 25,000, and 50,000 ppm groups for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle 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 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.

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 studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1988) (*cis* isomer) or Mortelmans *et al.* (1986) (*trans* and *cis,trans* isomers). The three isomers were sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). They were incubated with the *Salmonella typhimurium* tester strains TA97 (*cis* isomer only), TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of the chemical. For the study of *cis*-1,2-dichloroethylene conducted at SRI International and the study of *trans*-1,2-dichloroethylene, 10,000 µg/plate was selected as the high dose. For the study of *cis*-1,2-dichloroethylene conducted at Microbiological Associates, Inc., and the study of *cis,trans*-1,2-dichloroethylene, the high dose was limited by toxicity. For *trans*- and *cis,trans*-1,2-dichloroethylene, 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.

Chinese Hamster Ovary Cell Cytogenetics Protocols

Testing was performed as reported by Galloway *et al.* (1987). The three isomers were sent to the laboratories as coded aliquots by Radian Corporation. They were tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of the chemical. For the *cis,trans*-1,2-dichloroethylene trials conducted without S9, the high dose was limited by toxicity; in all other trials of each

chemical, the highest dose was limited by study design to 5,000 µg/mL (*cis* and *trans* isomers) or 12,630 µg/mL (*cis,trans* isomer). 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 *cis,trans*-1,2-dichloroethylene or 26 hours with *cis*- or *trans*-1,2-dichloroethylene in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 25.8 or 26 hours, the medium containing the chemical was 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 *cis*-, *trans*-, or *cis,trans*-1,2-dichloroethylene, serum-free medium, and S9 for 2 hours. The medium was then replaced with medium containing serum and BrdU and no test chemical. Incubation proceeded for an additional 25.5 to 26.5 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.

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 *cis*- or *trans*-1,2-dichloroethylene for 10 hours or with *cis,trans*-1,2-dichloroethylene for 20.5 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 *cis*-, *trans*-, or *cis,trans*-1,2-dichloroethylene S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 11 hours (*cis* and *trans* isomers) or 8.5 hours (*cis,trans* mixture) 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 of *cis,trans*-1,2-dichloroethylene was based on the cell cycle information obtained in the SCE test: because cell cycle delay was anticipated in the trial conducted without S9, the incubation period was extended.

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 (*cis,trans* mixture) or two hundred (*cis* and *trans* isomers) 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 resulted in an equivocal call (Galloway *et al.*, 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

Mouse Bone Marrow Cytogenetic Test Protocols

Sister Chromatid Exchange Test: A dose range-finding study was performed in the absence of adequate toxicity information from the literature, and the high dose was limited to 2,000 mg/kg. Male B6C3F$_1$ mice (five animals per dose group) were injected intraperitoneally with *cis*- or *trans*-1,2-dichloroethylene dissolved in corn oil. Vehicle control animals received equivalent injections of corn oil only. The positive control was dimethylbenzanthracene.

A standard harvest time of 23 hours was used. The animals were implanted subcutaneously with a BrdU tablet (McFee *et al.*, 1983) 24 hours before harvest (1 hour before *cis*- or *trans*-1,2-dichloroethylene treatment). The use of BrdU allowed selection of the appropriate cell population (cells in the second metaphase following *cis*- or *trans*-1,2-dichloroethylene treatment) for scoring. Two hours before sacrifice, the animals received an intraperitoneal injection of colchicine in saline. The animals were killed 23 hours after treatment (24 hours after BrdU dosing). One or both femurs were removed, and the marrow was flushed out with phosphate-buffered saline (pH 7.0). The cells were treated with a hypotonic salt solution, fixed, and dropped onto chilled slides. After a 24-hour drying period, the slides were stained with fluorescence-plus-Giemsa and scored.

Twenty-five second-division metaphase cells were scored from each of four animals per treatment group. Responses were evaluated as SCEs/cell, and the data were analyzed by a trend test (Margolin *et al.*, 1986).

Chromosomal Aberrations Test: A dose range-finding study was performed in the absence of adequate toxicity information from the literature, and the high dose was limited to 2,000 mg/kg. A standard harvest time of 17 hours was used.

Male B6C3F$_1$ mice (10 animals per dose group) were injected intraperitoneally with *cis*- or *trans*-1,2-dichloroethylene dissolved in corn oil. Vehicle control animals received equivalent injections of corn oil only. The positive control was dimethylbenzanthracene. The animals were subcutaneously implanted with a BrdU tablet (McFee *et al.*, 1983) 18 hours before the scheduled harvest (1 hour before injection of *cis*- or *trans*-1,2-dichloroethylene). The use of BrdU allowed selection of the appropriate cell population for scoring. (Abs induced by chemical administration are present in maximum number at the first metaphase following treatment; they decline in number during subsequent nuclear divisions due to cell death.) Two hours before sacrifice, the animals received an intraperitoneal injection of colchicine in saline. The animals were killed 17 hours after *cis*- or *trans*-1,2-dichloroethylene injection (18 hours after BrdU dosing). One or both femurs were removed, and the marrow was flushed out with phosphate-buffered saline (pH 7.0). Cells were treated with a hypotonic salt solution, fixed, and dropped onto chilled slides. After a 24-hour drying period, the slides were stained with fluorescence-plus-Giemsa and scored.

Fifty first-division metaphase cells were scored from each of seven or eight animals per treatment group. Responses were evaluated as the percentage of aberrant metaphase cells, excluding gaps. The data were analyzed by a trend test (Margolin *et al.*, 1986). The trend test P value must be less than or equal to 0.025 for a test to be significant; pairwise comparisons of each treatment group to the solvent control group are significant when P is less than or equal to 0.025 divided by the number of groups treated with *cis*- or *trans*-1,2-dichloroethylene.

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 10 animals per exposure group. In addition, 1,000 polychromatic erythrocytes (PCEs) were scored per animal to determine the percentage of PCEs in the total erythrocyte population.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-

Armitage trend test, followed by pairwise comparisons between each exposure group and the untreated control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). 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

All rats exposed to *trans*-1,2-dichloroethylene survived to the end of the study (Table 4). The final mean body weight and body weight gain of males in the 50,000 ppm group were significantly less than those of the vehicle controls (Table 4 and Figure 1). Feed consumption by the exposed groups was similar to that by the vehicle controls. Exposure concentrations of 3,125, 6,250, 12,500, 25,000, and 50,000 ppm resulted in average daily doses of 190, 380, 770, 1,540, and 3,210 mg *trans*-1,2-dichloroethylene per kg body weight for males and 190, 395, 780, 1,580, and 3,245 mg/kg for females. There were no clinical findings of toxicity. 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 *trans*-1,2-Dichloroethylene

Concentration (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	129 ± 3	362 ± 7	232 ± 5			
Vehicle Control	10/10	127 ± 3	360 ± 6	232 ± 5		14.3	15.3
3,125	10/10	128 ± 2	365 ± 5	237 ± 6	101	14.1	15.1
6,250	10/10	128 ± 3	361 ± 3	233 ± 3	100	14.5	15.8
12,500	10/10	127 ± 3	357 ± 5	230 ± 5	99	14.6	15.6
25,000	10/10	128 ± 2	350 ± 6	222 ± 5	97	14.3	15.5
50,000	10/10	127 ± 3	339 ± 4**	213 ± 3**	94	14.2	15.8
Female							
Untreated Control	10/10	111 ± 3	196 ± 3	86 ± 1			
Vehicle Control	10/10	111 ± 2	190 ± 4	79 ± 3		10.6	9.8
3,125	10/10	111 ± 2	198 ± 3	87 ± 2	104	10.9	9.8
6,250	10/10	110 ± 2	203 ± 2*	93 ± 2**	107	10.6	10.0
12,500	10/10	110 ± 2	198 ± 3	88 ± 2*	104	10.9	10.5
25,000	10/10	110 ± 2	196 ± 3	86 ± 2	103	10.8	9.8
50,000	10/10	110 ± 2	191 ± 2	81 ± 2	101	11.2	9.9

* Significantly different ($P \le 0.05$) from the vehicle control group by Dunnett's test
** Significantly different ($P \le 0.01$) from the vehicle control group by Williams' or Dunnett's test
[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 per animal per day.

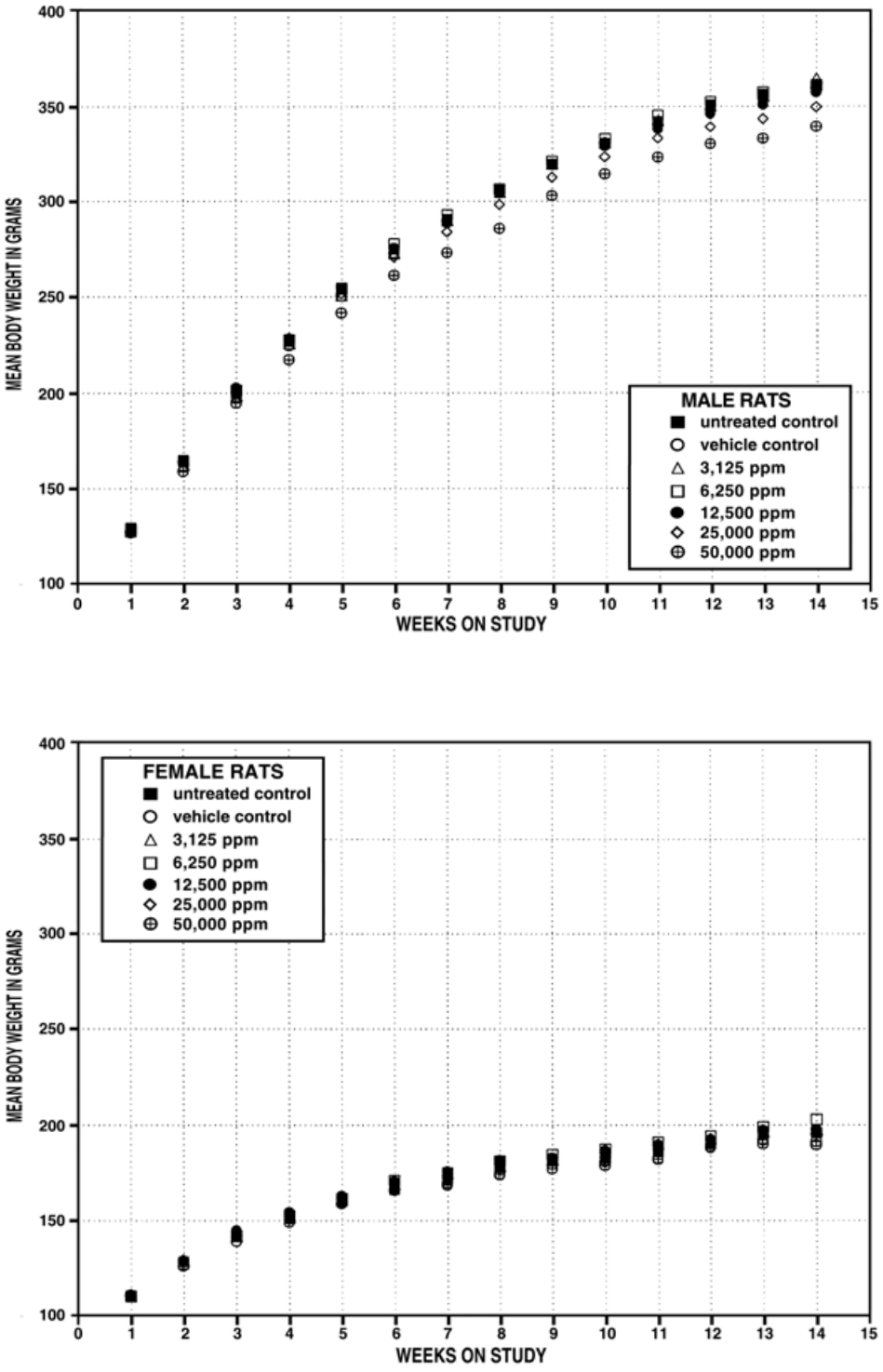


FIGURE 1
Body Weights of Male and Female Rats Exposed to *trans*-1,2-Dichloroethylene in Feed for 14 Weeks

The hematology data for rats are listed in Tables 5 and B1. On day 21 and at week 14, there were decreases in the circulating erythroid mass in exposed males and females, as evidenced by decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts. On day 21, these erythron effects occurred most consistently in the 25,000 and 50,000 ppm groups; at week 14, these effects were also observed in males exposed to 6,250 or 12,500 ppm. At both time points, the decrease in the erythron mass was of minimal severity, and, generally, the suppression was approximately 5% or less compared to the vehicle control values.

Females exposed to 12,500 ppm or greater had significantly decreased serum alkaline phosphatase activities compared to the vehicle controls on day 21 (Table B1). These decreases were minimal in severity, were no greater than approximately 13%, and were transient, with alkaline phosphatase activities in the affected groups returning to vehicle control levels by week 14. On day 21 and at week 14, there was minimal suppression of serum 5′-nucleotidase activities in males and females in the 50,000 ppm groups. There were sporadic differences in clinical chemistry parameters at various time points that generally did not demonstrate an exposure concentration relationship or were inconsistent between males and females; these differences were not considered to be toxicologically relevant.

The liver weights of female rats exposed to 6,250 ppm or greater were significantly greater than those of the vehicle controls (Table C1). Males in the 25,000 and 50,000 ppm groups had significantly lower absolute kidney weights than the vehicle control group.

Sperm motility and vaginal cytology parameters of exposed rats were generally similar to those of the vehicle controls (Tables D1 and D2). No gross or microscopic lesions were observed that could be attributed to *trans*-1,2-dichloroethylene exposure (Tables A1 and A2).

TABLE 5
Selected Hematology Data for Rats in the 14-Week Feed Study of *trans*-1,2-Dichloroethylene[a]

	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
n						
Day 5	10	10	10	10	10	10
Day 21	10	10	9	7	9	10
Week 14	10	10	9	10	10	10
Hematocrit (%)						
Day 5	38.8 ± 0.4	39.1 ± 0.6	39.5 ± 0.7	40.5 ± 0.9	39.9 ± 0.6	39.8 ± 0.6
Day 21	43.3 ± 0.4	43.1 ± 0.5	42.4 ± 0.2	42.5 ± 0.6	40.9 ± 1.7	41.6 ± 0.4*
Week 14	41.1 ± 0.3	41.4 ± 0.3	40.4 ± 0.4	39.9 ± 0.4*	39.8 ± 0.3*	39.0 ± 0.8*
Manual hematocrit (%)						
Day 5	42.8 ± 0.4	43.2 ± 0.6	43.5 ± 0.7	44.6 ± 0.8	44.1 ± 0.5	43.8 ± 0.6
Day 21	49.1 ± 0.6	48.3 ± 0.8	47.8 ± 0.4	48.4 ± 0.5	46.1 ± 1.8	47.1 ± 0.6
Week 14	45.0 ± 0.4	45.2 ± 0.2	44.2 ± 0.4	44.1 ± 0.4	43.5 ± 0.4*	43.2 ± 0.8*
Hemoglobin (g/dL)						
Day 5	14.6 ± 0.2	14.7 ± 0.2	14.8 ± 0.2	15.2 ± 0.3	14.9 ± 0.2	14.8 ± 0.1
Day 21	15.9 ± 0.2	15.7 ± 0.3	15.4 ± 0.1	15.6 ± 0.1	14.9 ± 0.6*	15.1 ± 0.2**
Week 14	14.8 ± 0.1	15.0 ± 0.1	14.7 ± 0.1	14.5 ± 0.1	14.5 ± 0.1	14.3 ± 0.3
Erythrocytes (10^6/μL)						
Day 5	6.50 ± 0.07	6.55 ± 0.09	6.61 ± 0.13	6.78 ± 0.15	6.68 ± 0.09	6.71 ± 0.09
Day 21	7.13 ± 0.08	7.10 ± 0.09	6.91 ± 0.04*	6.96 ± 0.09	6.69 ± 0.25*	6.82 ± 0.07**
Week 14	8.14 ± 0.08	8.17 ± 0.05	7.93 ± 0.10*	7.84 ± 0.09*	7.79 ± 0.08**	7.56 ± 0.15**
Female						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 5	41.2 ± 0.6	41.1 ± 0.6	40.3 ± 0.6	41.2 ± 0.5	41.0 ± 0.4	40.9 ± 0.5
Day 21	45.4 ± 0.4	45.2 ± 0.3	44.0 ± 0.3	44.6 ± 0.3	43.7 ± 0.5	43.9 ± 0.8
Week 14	41.9 ± 0.3	41.5 ± 0.5	41.2 ± 0.5	41.2 ± 0.2	40.4 ± 0.3**	40.1 ± 0.4**
Manual hematocrit (%)						
Day 5	44.8 ± 0.6	45.1 ± 0.6	43.7 ± 0.7	44.6 ± 0.6	44.3 ± 0.4	44.7 ± 0.5
Day 21	49.2 ± 0.5	49.0 ± 0.4	47.5 ± 0.4	48.3 ± 0.4	47.6 ± 0.5	48.0 ± 0.6
Week 14	44.9 ± 0.3	44.6 ± 0.4	44.1 ± 0.4	44.3 ± 0.2	43.6 ± 0.3**	43.3 ± 0.4**
Hemoglobin (g/dL)						
Day 5	15.1 ± 0.2	15.3 ± 0.2	14.9 ± 0.2	15.2 ± 0.2	15.1 ± 0.1	15.1 ± 0.2
Day 21	16.6 ± 0.1	16.6 ± 0.2	16.1 ± 0.2*	16.4 ± 0.1	15.9 ± 0.2**	16.1 ± 0.2*
Week 14	15.2 ± 0.1	15.2 ± 0.2	15.0 ± 0.1	15.0 ± 0.1	14.7 ± 0.1**	14.5 ± 0.1**
Erythrocytes (10^6/μL)						
Day 5	7.00 ± 0.13	7.00 ± 0.13	6.83 ± 0.13	6.95 ± 0.10	6.92 ± 0.09	6.92 ± 0.10
Day 21	7.52 ± 0.08	7.48 ± 0.06	7.28 ± 0.07*	7.34 ± 0.06*	7.21 ± 0.08**	7.21 ± 0.14*
Week 14	7.59 ± 0.06	7.58 ± 0.10	7.50 ± 0.08	7.49 ± 0.04	7.34 ± 0.05**	7.20 ± 0.08**

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

MICE

There were no treatment-related deaths of mice exposed to *trans*-1,2-dichloroethylene (Table 6). The final mean body weights and body weight gains of males and females in the 50,000 ppm groups and the body weight gains of females in the 12,500 and 25,000 ppm groups were significantly less than those of the vehicle controls (Table 6 and Figure 2). Feed consumption by the exposed groups was similar to that by the vehicle controls. Exposure concentrations of 3,125, 6,250, 12,500, 25,000, and 50,000 ppm resulted in average daily doses of 480, 920, 1,900, 3,850, and 8,065 mg/kg for males and 450, 915, 1,830, 3,760, and 7,925 mg/kg for females. There were no clinical findings of toxicity. Results of the functional observation battery indicated no exposure-related findings of neurotoxicity.

TABLE 6
Survival, Body Weights, and Feed Consumption of Mice in the 14-Week Feed Study of *trans*-1,2-Dichloroethylene

Concentration (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	20.0 ± 0.4	30.6 ± 0.9	10.6 ± 0.5			
Vehicle Control	10/10	19.5 ± 0.4	30.3 ± 0.6	10.8 ± 0.5		3.7	4.2
3,125	10/10	20.1 ± 0.3	29.8 ± 0.4	9.7 ± 0.3	98	4.0	4.2
6,250	10/10	19.7 ± 0.4	30.5 ± 0.6	10.8 ± 0.7	101	3.7	3.9
12,500	10/10	20.0 ± 0.4	29.2 ± 1.0	9.2 ± 0.9	96	3.7	4.1
25,000	10/10	19.3 ± 0.6	29.0 ± 0.5	9.7 ± 0.6	96	3.7	4.1
50,000	10/10	20.0 ± 0.3	28.2 ± 0.5*	8.1 ± 0.5**	93	3.8	4.2
Female							
Untreated Control	8/10[d]	16.8 ± 0.4	24.3 ± 0.5	7.1 ± 0.4			
Vehicle Control	10/10	15.9 ± 0.4	23.3 ± 0.3	7.4 ± 0.4		3.1	3.1
3,125	10/10	16.2 ± 0.4	23.8 ± 0.5	7.6 ± 0.3	102	2.9	3.2
6,250	9/10[d]	17.1 ± 0.6	25.0 ± 0.6	8.0 ± 0.5	108	3.1	3.3
12,500	10/10	16.4 ± 0.4	22.0 ± 0.4	5.6 ± 0.2**	94	2.8	3.2
25,000	9/10[d]	16.1 ± 0.4	22.3 ± 0.4	6.0 ± 0.3**	96	3.0	3.1
50,000	9/10[d]	16.2 ± 0.4	21.7 ± 0.4*	5.4 ± 0.3**	93	2.6	3.3

* Significantly different ($P \le 0.05$) from the vehicle control group by Williams' test
** $P \le 0.01$
[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 per animal per day.
[d] Week of death: 14 (accidental deaths)

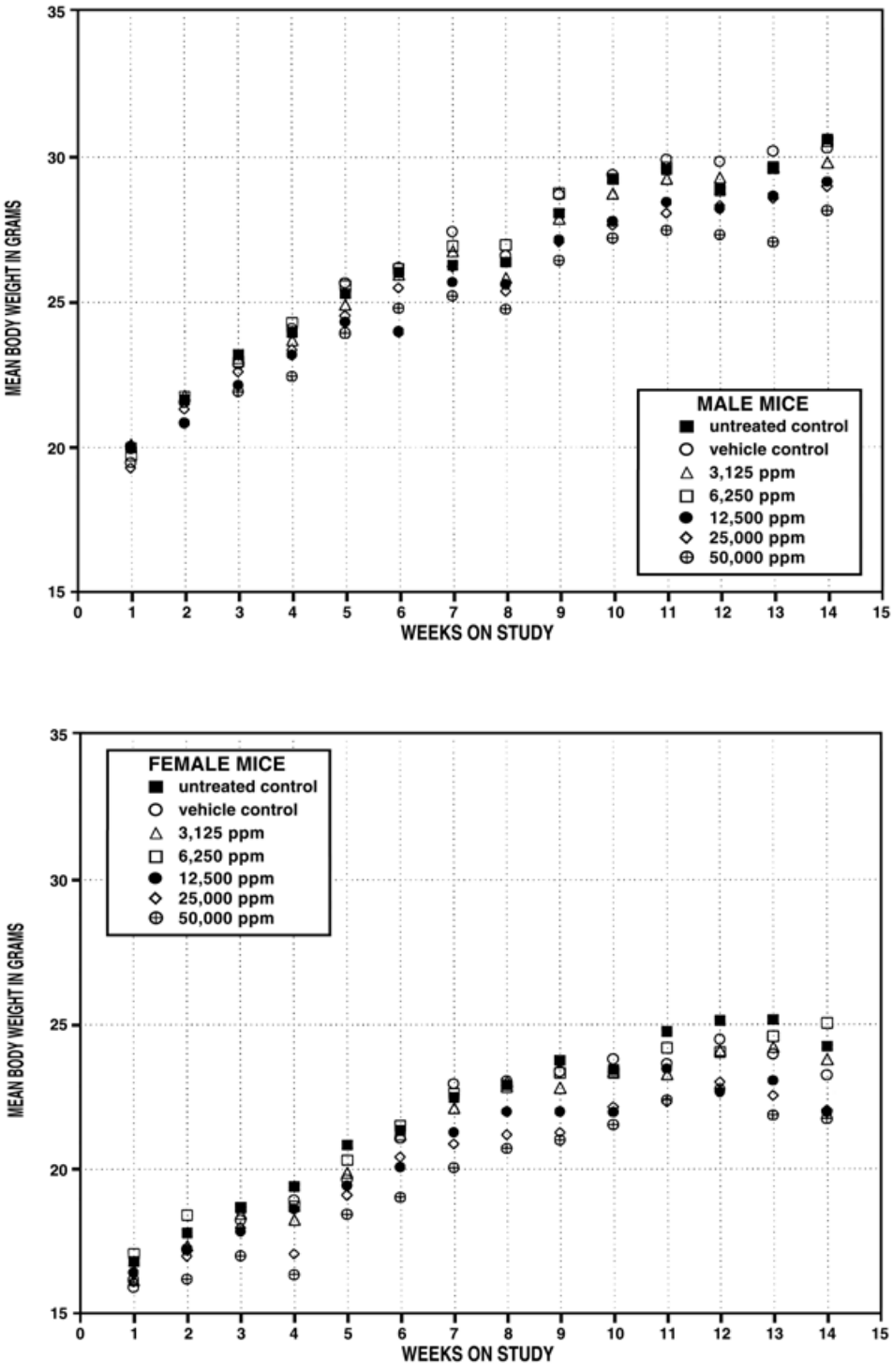


FIGURE 2
Body Weights of Male and Female Mice Exposed to *trans*-1,2-Dichloroethylene in Feed for 14 Weeks

No alterations in clinical chemistry parameters for mice were attributed to exposure (Table B2). The relative liver weights of males exposed to 12,500 ppm or greater and females exposed to 25,000 or 50,000 ppm were significantly greater than those of the vehicle controls (Table C2). Sperm motility and vaginal cytology parameters of exposed mice were similar to those of the vehicle controls (Tables D3 and D4). No gross or microscopic lesions were observed that could be attributed to *trans*-1,2-dichloroethylene exposure (Tables A3 and A4).

GENETIC TOXICOLOGY

cis-1,2-Dichloroethylene, tested at two laboratories, was not mutagenic in any of five strains of *Salmonella typhimurium* with or without induced hamster or rat S9 metabolic activation enzymes (Table E1; Zeiger *et al.*, 1988). Neither *trans*- nor *cis,trans*-1,2-dichloroethylene was mutagenic in any of four strains of *S. typhimurium* (Tables E2 and E3; Mortelmans *et al.*, 1986). In Chinese hamster ovary (CHO) cells *in vitro*, *cis*-1,2-dichloroethylene induced sister chromatid exchanges (SCEs) in the absence of S9 at concentrations of 500 to 5,000 µg/mL (Table E4); with S9, results from a single trial were judged to be equivocal, because although a clear dose-related increase in SCEs was observed (P=0.001), none of the individual dose points differed significantly from the solvent control value. *trans*-1,2-Dichloroethylene did not induce SCEs without S9; the results of a single trial with S9 were judged to be equivocal based on the trend test (P<0.005) and the absence of significant increases (≥20%) at any of the individual dose points (Table E5). A strongly positive response was obtained for SCE induction by *cis,trans*-1,2-dichloroethylene at all doses tested, with and without S9 metabolic activation enzymes (Table E6). No induction of chromosomal aberrations (Abs) by *cis*-, *trans*-, or *cis,trans*-1,2-dichloroethylene was observed in cultured CHO cells, with or without S9 (Tables E7, E8, and E9). For the *cis,trans* isomer, intermediate toxicity occurred at some of the doses tested in the absence of S9, and etching of the plastic tissue culture flasks occurred at the higher doses with and without S9. In the first Abs trial with S9, an increase in Abs was noted at the lowest *cis,trans*-1,2-dichloroethylene dose tested (772 µg/mL); although the increase was not statistically significant, a second trial was performed using similar doses. Again, the lowest dose tested (455 µg/mL) in this trial produced the greatest increase in Abs, but the difference was not significant. Thus, the Abs test for *cis,trans*-1,2-dichloroethylene was judged to be negative.

In vivo, neither *cis*- nor *trans*-1,2-dichloroethylene administered by intraperitoneal injection at doses of 500 to 2,000 mg/kg induced SCEs or Abs in bone marrow cells of male mice in tests using the standard sampling times of 23 hours (SCE test) or 17 hours (Abs test) (Tables E10 through E13). Experiments using an extended harvest time to compensate for special metabolic requirements or induced cell cycle delays are required before the negative results in the *in vivo* bone marrow tests may be considered complete. *trans*-1,2-Dichloroethylene

administered in microcapsules in feed for 14 weeks did not increase the frequency of micronucleated normochromatic erythrocytes (NCEs) in the peripheral blood of male or female mice (Table E14). In addition, no effect on the percentage of micronucleated polychromatic erythrocytes among the total erythrocyte population was observed, indicating no inhibition or stimulation of erythropoiesis in the bone marrow of exposed mice.

DISCUSSION

Toxicology studies of *trans*-1,2-dichloroethylene were conducted by oral administration to F344/N rats and B6C3F$_1$ mice for 14 weeks. *trans*-1,2-Dichloroethylene was administered in microcapsules in the feed. This method prolongs chemical stability in rodent diets and is not toxic (Dieter *et al.*, 1993; NTP, 2000, 2002). Microencapsulation allowed continual ingestion of *trans*-1,2-dichloroethylene, was a more appropriate route than oral gavage, and allowed for higher exposure concentrations than would be obtainable in drinking water. Two control groups were included in these studies to detect any adverse effects of ingestion of microcapsules. No biologically significant differences in body or organ weights were observed between the untreated and vehicle controls. Because there were no differences in response between the control groups, statistical comparisons only between the vehicle controls and the exposed groups are presented. Previous work has shown that the bioavailability of chemical administered in microcapsules was comparable to that when administered by gavage (Yuan *et al.*, 1993; Hébert *et al.*, 1994).

Because 1,2-dichloroethylene occurs in two isomeric forms, *cis* and *trans*, 15-day pilot studies using each isomer and a mixture of the two isomers in microcapsules in feed were performed to identify the most biologically active isomer and to aid in exposure concentration selection for the 14-week studies. The maximum allowable concentration of 50,000 ppm 1,2-dichloroethylene was used as the highest exposure concentration in the 15-day pilot studies. No chemical-related lesions were observed in rats or mice in any exposure group. Kidney and liver weights were increased in rats and mice exposed to *cis*- or *trans*-1,2-dichloroethylene but not in animals exposed to a mixture of *cis*- and *trans*-1,2-dichloroethylene (data not shown). Large variations in initial mean body weights of rats exposed to *cis*-1,2-dichloroethylene confounded interpretation of body and organ weight data. The most significant effect was a 20% reduction in final mean body weights of male rats exposed to *trans*-1,2-dichloroethylene. Based on these preliminary data and because of its prevalence in industrial applications, the *trans* isomer was selected for 14-week studies. Due to the low toxicity observed in the 15-day pilot studies, 50,000 ppm *trans*-1,2-dichloroethylene was selected as the highest concentration for use in the 14-week studies.

In general, toxic effects caused by ingestion of *trans*-1,2-dichloroethylene for 14 weeks were minimal. The liver appeared to be the target organ for *trans*-1,2-dichloroethylene toxicity based on organ weight data and has been identified as such in several studies (Freundt *et al.*, 1977; Hayes *et al.*, 1987; McCauley *et al.*, 1995). In the

present study and that by Hayes *et al.* (1987), no gross or microscopic lesions were observed in animals exposed to *trans*-1,2-dichloroethylene for 14 weeks. Increased liver weights are often sensitive indicators for toxicity, especially when accompanied by gross or microscopic lesions. In the case of *trans*-1,2-dichloroethylene, lesions were observed only in one study in which inhalation exposure caused fatty accumulation in the liver and Kupffer cells (Freundt *et al.*, 1977). These lesions were interpreted to be a result of inhibition of cytochrome P450 enzymes by the *trans* isomer. In the present studies and in those of McCauley *et al.* (1995) and Hayes *et al.* (1987), histopathologic lesions did not accompany increased liver weights. Liver weight changes following chemical insult are not uncommon and are often associated with proliferation of the smooth endoplasmic reticulum and increased cytochrome P450 content or drug metabolizing enzyme activity, which can represent adaptive and/or toxic responses (de la Iglesia *et al.*, 1982).

In the current studies, modest decreases in absolute kidney weights occurred in male rats exposed to 25,000 or 50,000 ppm *trans*-1,2-dichloroethylene with no accompanying lesions or changes in clinical chemistry parameters. Changes in kidney weights, with no gross or microscopically visible lesions, also have been observed by Freundt *et al.* (1977), Hayes *et al.* (1987), and McCauley *et al.* (1995). McCauley *et al.* (1995) observed a decrease in blood urea nitrogen levels in male and female rats exposed to *cis*-1,2-dichloroethylene for 14 days, but this change was not dose related, and its toxicological significance was unknown. Decreased kidney weights, although observed in the current studies and previous studies, do not provide information on kidney toxicity in the absence of histopathologic or clinical evidence of toxicity.

In the current study, slight, but generally exposure concentration-dependent, decreases in red cell mass were observed in male rats exposed to 6,250 ppm or greater and female rats exposed to 25,000 or 50,000 ppm. In male and female Sprague-Dawley rats exposed to 872 mg/kg *cis*-1,2-dichloroethylene by gavage for 90 days, significant decreases in the circulating erythroid mass were observed; however, this response was not dose related and was not considered to be biologically relevant (McCauley *et al.*, 1995). No hematologic response was observed in male or female CD-1 mice exposed to 2,000 mg/L *trans*-1,2-dichloroethylene (daily dose approximately 387 mg/kg) in drinking water for 90 days (Barnes *et al.*, 1985). The *cis* and *trans* isomers may have an effect on hematologic endpoints; however, more consistency between studies is necessary before the biological significance (if any) is known.

trans-1,2-Dichloroethylene is a mechanism-based inhibitor of CYP2E1 and is converted by CYP2E1 to an epoxide intermediate that subsequently binds to the heme moiety and as such is the rate-limiting step of *trans*-1,2-dichloroethylene metabolism *in vivo*. The exposure concentrations of *trans*-1,2-dichloroethylene in the present studies are greater than those required to suppress CYP2E1 after single or repeated gavage

administration (Mathews *et al.*, 1997). Although CYP2E1 activity was not assessed in the present study, it is likely that its activity was inhibited, thus affecting the metabolism of *trans*-1,2-dichloroethylene.

trans-1,2-Dichloroethylene has been shown to cause central nervous system effects in humans, characterized by dizziness, drowsiness, vertigo, and increased intracranial pressure (ATSDR, 1990). To address the possible central nervous system depression effects, a functional observation battery was included in the current studies. The results suggest that ingestion of *trans*-1,2-dichloroethylene does not cause central nervous system depression in rats or mice. Other investigators have observed ataxia and loss of righting reflex; however, these effects were observed at lethal doses of *cis*- or *trans*-1,2-dichloroethylene (Munson *et al.*, 1982; Hayes *et al.*, 1987; McCauley *et al.*, 1995).

Symmetrically substituted oxiranes such as 1,2-dichloroethylene and 1,1,2,2-tetrachloroethylene are more stable and less mutagenic than unsymmetrical chlorinated oxiranes such as 1,1-dichloroethylene, 1,1,2-trichloroethylene, and vinyl chloride (Henschler *et al.*, 1976; Cantelli-Forti and Bronzetti, 1988). However, the carcinogenicity of chlorinated hydrocarbons may or may not lie in the reactivity of the epoxide intermediate. Of the chlorinated hydrocarbons, it has been reported that 1,1-dichloroethylene (Chu and Milman, 1981), vinyl chloride (Apfeldorf and Infante, 1981; Maltoni *et al.*, 1981; Rice, 1981), trichloroethylene (NCI, 1976; Fukuda *et al.*, 1983; NTP, 1988, 1990), and tetrachloroethylene (NCI, 1977; NTP, 1986) are carcinogens *in vivo*. From the literature, the carcinogenicity of 1,1-dichloroethylene is primarily associated with inhalation exposure (Lee *et al.*, 1978; Chu and Milman, 1981). But, in the case of vinyl chloride (Apfeldorf and Infante, 1981; Infante, 1981; Maltoni *et al.*, 1981; Rice, 1981), trichloroethylene (NCI, 1976; Fukuda *et al.*, 1983; NTP, 1988, 1990), and tetrachloroethylene (NTP, 1986), carcinogenicity occurs through both the inhalation and oral routes (NCI, 1977; NTP, 1986). 1,2-Dichloroethylene may be similarly toxic via inhalation. Freundt *et al.* (1977) reported severe alveolar septal distention and pulmonary hyperemia in rat lungs after inhalation exposure to *trans*-1,2-dichloroethylene at concentrations greater than or equal to the current threshold limit value of 200 ppm (ACGIH, 2000).

In summary, very little toxicity was associated with ingestion of microencapsulated *trans*-1,2-dichloroethylene. Histopathology and clinical chemistry data, combined with body and organ weight data, revealed that the maximum tolerated dose was not reached in these studies. The lowest exposure concentration used in these studies, 3,125 ppm, exceeds the current drinking water limits of 70 to 100 ppb by a large factor.

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APPENDIX A
SUMMARY OF LESIONS
IN RATS AND MICE

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

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Survivors							
Terminal sacrifice	10	10	10	10	10	10	10
Animals examined microscopically	10	10	1		2		10
Alimentary System							
Liver	(10)	(10)	(1)		(2)		(10)
Hepatodiaphragmatic nodule			1 (100%)		1 (50%)		
Inflammation, chronic	1 (10%)						
Pancreas	(10)	(10)					(10)
Acinus, atrophy, focal	3 (30%)	2 (20%)					
Cardiovascular System							
Heart	(10)	(10)					(10)
Cardiomyopathy	9 (90%)	6 (60%)					7 (70%)
Pigmentation	1 (10%)						
Endocrine System							
Thyroid gland	(10)	(10)					(10)
Ultimobranchial cyst	2 (20%)	2 (20%)					
General Body System							
None							
Genital System							
Preputial gland	(10)	(10)					(9)
Inflammation, chronic	6 (60%)	2 (20%)					1 (11%)
Inflammation, chronic active		1 (10%)					
Bilateral, inflammation, chronic	2 (20%)	3 (30%)					2 (22%)
Testes	(10)	(10)					(10)
Germinal epithelium, atrophy		1 (10%)					
Hematopoietic System							
Lymph node, mandibular	(10)	(10)					(10)
Hemorrhage							2 (20%)
Lymph node, mesenteric	(10)	(10)					(10)
Infiltration cellular, histiocyte	1 (10%)						3 (30%)
Spleen	(10)	(10)					(10)
Capsule, inflammation, chronic, focal	1 (10%)						
Thymus	(10)	(10)					(9)
Hemorrhage	3 (30%)	1 (10%)					1 (11%)

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

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Integumentary System							
None							
Musculoskeletal System							
None							
Nervous System							
None							
Respiratory System							
Lung	(10)	(10)					(10)
Hemorrhage	2 (20%)	1 (10%)					3 (30%)
Interstitium, inflammation, chronic	1 (10%)	1 (10%)					3 (30%)
Interstitium, inflammation, chronic active		1 (10%)					
Nose	(10)	(10)					
Fungus	1 (10%)						
Inflammation, chronic active	1 (10%)						
Special Senses System							
None							
Urinary System							
Kidney	(10)	(10)					(10)
Nephropathy, chronic		3 (30%)					4 (40%)
Bilateral, nephropathy, chronic	9 (90%)	7 (70%)					4 (40%)

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

TABLE A2
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of *trans*-1,2-Dichloroethylene[a]

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Survivors							
Terminal sacrifice	10	10	10	10	10	10	10
Animals examined microscopically	10	10	5	5	3	2	10
Alimentary System							
Liver	(10)	(10)	(2)		(1)	(1)	(10)
Hepatodiaphragmatic nodule	1 (10%)	2 (20%)	1 (50%)		1 (100%)		2 (20%)
Inflammation, chronic	1 (10%)						
Pancreas	(10)	(10)					(10)
Acinus, atrophy, focal	2 (20%)						
Cardiovascular System							
Heart	(10)	(10)					(10)
Cardiomyopathy	2 (20%)	1 (10%)					2 (20%)
Endocrine System							
Pituitary gland	(10)	(10)					(10)
Pars distalis, cyst	1 (10%)	1 (10%)					
Thyroid gland	(10)	(10)					(10)
Ultimobranchial cyst		1 (10%)					
General Body System							
None							
Genital System							
Clitoral gland	(10)	(10)					(10)
Infiltration cellular, chronic							1 (10%)
Inflammation, chronic	1 (10%)	3 (30%)					1 (10%)
Bilateral, inflammation, chronic	2 (20%)	3 (30%)					2 (20%)
Ovary	(10)	(10)	(2)	(1)	(1)		(10)
Cyst			2 (100%)	1 (100%)	1 (100%)		1 (10%)
Uterus	(10)	(10)	(1)	(4)	(2)	(1)	(10)
Hydrometra	2 (20%)	3 (30%)	1 (100%)	4 (100%)	2 (100%)	1 (100%)	4 (40%)
Endometrium, polyp stromal	1 (10%)						
Hematopoietic System							
Bone marrow	(10)	(10)					(10)
Atrophy	1 (10%)						
Inflammation, granulomatous	4 (40%)	2 (20%)					2 (20%)
Myelofibrosis	1 (10%)						3 (30%)
Lymph node, mandibular	(10)	(10)					(10)
Hemorrhage		1 (10%)					

TABLE A2
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of *trans*-1,2-Dichloroethylene

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Hematopoietic System (continued)							
Lymph node, mesenteric	(10)	(10)					(10)
Hyperplasia, lymphoid	1 (10%)						
Infiltration cellular, histiocyte	5 (50%)	2 (20%)					3 (30%)
Spleen	(10)	(10)	(1)				(10)
Capsule, fibrosis			1 (100%)				
Thymus	(10)	(10)					(10)
Hemorrhage	1 (10%)						1 (10%)
Integumentary System							
None							
Musculoskeletal System							
None							
Nervous System							
None							
Respiratory System							
Lung	(10)	(10)					(10)
Hemorrhage							1 (10%)
Interstitium, inflammation, chronic	1 (10%)						4 (40%)
Nose	(10)	(10)					(10)
Inflammation, chronic	1 (10%)						1 (10%)
Special Senses System							
None							
Urinary System							
Kidney	(10)	(10)					(10)
Nephropathy, chronic	2 (20%)						
Bilateral, mineralization, focal	10 (100%)	10 (100%)					10 (100%)

[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 *trans*-1,2-Dichloroethylene[a]

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Survivors							
Terminal sacrifice	10	10	10	10	10	10	10
Animals examined microscopically	10	10					10
Alimentary System							
Gallbladder	(9)	(10)					(9)
Cyst		1 (10%)					
Stomach, glandular	(10)	(10)					(10)
Cyst	2 (20%)						1 (10%)
Cardiovascular System							
Heart	(10)	(10)					(10)
Valve, pigmentation	1 (10%)						
Endocrine System							
Adrenal cortex	(10)	(10)					(10)
Capsule, hyperplasia	1 (10%)	1 (10%)					1 (10%)
Adrenal medulla	(10)	(10)					(10)
Angiectasis	1 (10%)	2 (20%)					2 (20%)
General Body System							
None							
Genital System							
Preputial gland	(10)	(10)					(10)
Atrophy	9 (90%)	6 (60%)					7 (70%)
Hematopoietic System							
Lymph node, mandibular	(10)	(9)					(10)
Hemorrhage		1 (11%)					
Spleen	(10)	(10)					(10)
Hematopoietic cell proliferation		1 (10%)					1 (10%)
Integumentary System							
None							
Musculoskeletal System							
None							

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Feed Study of *trans*-1,2-Dichloroethylene

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Nervous System							
None							
Respiratory System							
None							
Special Senses System							
None							
Urinary System							
Kidney	(10)	(10)					(10)
Inflammation, focal							1 (10%)

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

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

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Disposition Summary							
Animals initially in study	10	10	10	10	10	10	10
Survivors							
Accidental deaths	2			1		1	1
Terminal sacrifice	8	10	10	9	10	9	9
Animals examined microscopically	10	10			1	2	10
Alimentary System							
Liver	(10)	(10)			(1)		(10)
Inflammation, focal	3 (30%)	3 (30%)					
Necrosis					1 (100%)		1 (10%)
Stomach, glandular	(10)	(10)					(10)
Cyst	1 (10%)						
Cardiovascular System							
None							
Endocrine System							
Adrenal cortex	(10)	(10)					(10)
Capsule, hyperplasia	9 (90%)	9 (90%)					9 (90%)
Adrenal medulla	(10)	(10)					(10)
Angiectasis		1 (10%)					
Thyroid gland	(10)	(10)					
Ectopic tissue	1 (10%)						
General Body System							
None							
Genital System							
Clitoral gland	(7)	(9)					(6)
Atrophy	4 (57%)	7 (78%)					3 (50%)
Ovary	(10)	(10)				(1)	(10)
Hemorrhage						1 (100%)	
Hematopoietic System							
Lymph node, mandibular	(10)	(10)					(10)
Hemorrhage	1 (10%)	1 (10%)					1 (10%)
Spleen	(10)	(10)					(10)
Hematopoietic cell proliferation	1 (10%)	3 (30%)					6 (60%)
Integumentary System							
None							

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Feed Study of *trans*-1,2-Dichloroethylene

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Musculoskeletal System							
None							
Nervous System							
None							
Respiratory System							
Lung	(10)	(10)					(10)
Infiltration cellular, focal, lymphocyte	2 (20%)	5 (50%)					
Special Senses System							
None							
Urinary System							
None							

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

APPENDIX B
CLINICAL PATHOLOGY RESULTS

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

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male							
Hematology							
n							
Day 5	10	10	10	10	10	10	10
Day 21	9	10	10	9	7	9	10
Week 14	10	10	10	9	10	10	10
Automated hematocrit (%)							
Day 5	40.1 ± 1.0	38.8 ± 0.4	39.1 ± 0.6	39.5 ± 0.7	40.5 ± 0.9	39.9 ± 0.6	39.8 ± 0.6
Day 21	43.5 ± 0.5	43.3 ± 0.4	43.1 ± 0.5	42.4 ± 0.2	42.5 ± 0.6	40.9 ± 1.7	41.6 ± 0.4*
Week 14	41.9 ± 0.3	41.1 ± 0.3	41.4 ± 0.3	40.4 ± 0.4	39.9 ± 0.4*	39.8 ± 0.3*	39.0 ± 0.8*
Manual hematocrit (%)							
Day 5	44.0 ± 1.1	42.8 ± 0.4	43.2 ± 0.6	43.5 ± 0.7	44.6 ± 0.8	44.1 ± 0.5	43.8 ± 0.6
Day 21	49.0 ± 0.9	49.1 ± 0.6	48.3 ± 0.8	47.8 ± 0.4	48.4 ± 0.5	46.1 ± 1.8	47.1 ± 0.6
Week 14	45.2 ± 0.3	45.0 ± 0.4	45.2 ± 0.2	44.2 ± 0.4	44.1 ± 0.4	43.5 ± 0.4*	43.2 ± 0.8*
Hemoglobin (g/dL)							
Day 5	14.9 ± 0.3	14.6 ± 0.2	14.7 ± 0.2	14.8 ± 0.2	15.2 ± 0.3	14.9 ± 0.2	14.8 ± 0.1
Day 21	15.7 ± 0.2	15.9 ± 0.2	15.7 ± 0.3	15.4 ± 0.1	15.6 ± 0.1	14.9 ± 0.6*	15.1 ± 0.2**
Week 14	15.1 ± 0.1	14.8 ± 0.1	15.0 ± 0.1	14.7 ± 0.1	14.5 ± 0.1	14.5 ± 0.1	14.3 ± 0.3
Erythrocytes (10^6/µL)							
Day 5	6.70 ± 0.17	6.50 ± 0.07	6.55 ± 0.09	6.61 ± 0.13	6.78 ± 0.15	6.68 ± 0.09	6.71 ± 0.09
Day 21	7.09 ± 0.09	7.13 ± 0.08	7.10 ± 0.09	6.91 ± 0.04*	6.96 ± 0.09	6.69 ± 0.25*	6.82 ± 0.07**
Week 14	8.13 ± 0.07	8.14 ± 0.08	8.17 ± 0.05	7.93 ± 0.10*	7.84 ± 0.09*	7.79 ± 0.08**	7.56 ± 0.15**
Reticulocytes (10^6/µL)							
Day 5	0.19 ± 0.02	0.21 ± 0.02	0.23 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.21 ± 0.02	0.22 ± 0.02
Day 21	0.16 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01
Week 14	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Nucleated erythrocytes (10^3/µL)							
Day 5	0.90 ± 0.31	1.10 ± 0.35	1.50 ± 0.40	0.90 ± 0.23	0.90 ± 0.35	1.30 ± 0.21	1.10 ± 0.41
Day 21	0.33 ± 0.33	0.50 ± 0.22	0.20 ± 0.20	1.11 ± 0.31	1.29 ± 0.36	0.67 ± 0.24	0.40 ± 0.22
Week 14	0.30 ± 0.21	0.40 ± 0.27	0.10 ± 0.10	0.11 ± 0.11	0.40 ± 0.22	0.20 ± 0.13	0.20 ± 0.13
Mean cell volume (fL)							
Day 5	59.8 ± 0.3	59.6 ± 0.2	59.7 ± 0.4	59.8 ± 0.3	59.8 ± 0.2	59.7 ± 0.3	59.3 ± 0.2
Day 21	61.3 ± 0.3	60.8 ± 0.3	60.8 ± 0.2	61.3 ± 0.3	61.1 ± 0.7	61.2 ± 0.5	61.0 ± 0.3
Week 14	51.5 ± 0.2	50.5 ± 0.1	50.7 ± 0.1	50.9 ± 0.1*	50.9 ± 0.1*	51.1 ± 0.2*	51.6 ± 0.2**
Mean cell hemoglobin (pg)							
Day 5	22.3 ± 0.2	22.4 ± 0.1	22.4 ± 0.1	22.4 ± 0.1	22.4 ± 0.1	22.2 ± 0.2	22.0 ± 0.2
Day 21	22.2 ± 0.2	22.3 ± 0.1	22.2 ± 0.1	22.3 ± 0.1	22.4 ± 0.2	22.2 ± 0.2	22.1 ± 0.1
Week 14	18.5 ± 0.1	18.2 ± 0.1	18.3 ± 0.1	18.5 ± 0.2	18.5 ± 0.1	18.6 ± 0.2*	18.9 ± 0.1**
Mean cell hemoglobin concentration (g/dL)							
Day 5	37.3 ± 0.2	37.6 ± 0.1	37.5 ± 0.2	37.5 ± 0.2	37.5 ± 0.3	37.2 ± 0.1	37.2 ± 0.2
Day 21	36.2 ± 0.2	36.6 ± 0.2	36.5 ± 0.2	36.4 ± 0.1	36.7 ± 0.3	36.4 ± 0.2	36.3 ± 0.1
Week 14	36.0 ± 0.1	36.0 ± 0.1	36.2 ± 0.2	36.3 ± 0.3	36.3 ± 0.1	36.5 ± 0.2	36.6 ± 0.2*
Platelets (10^3/µL)							
Day 5	946.7 ± 27.7	923.0 ± 9.7	955.5 ± 13.7	887.0 ± 18.4	939.6 ± 24.5	913.8 ± 14.2	930.2 ± 10.4
Day 21	869.4 ± 11.5	863.5 ± 15.3	832.5 ± 14.1	848.6 ± 18.5	865.9 ± 11.9	883.9 ± 18.3	845.2 ± 16.2
Week 14	732.5 ± 12.8	727.1 ± 9.2	744.0 ± 10.5	713.7 ± 18.3	733.3 ± 14.5	724.1 ± 11.2	718.1 ± 17.9

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

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male (continued)							
Hematology (continued)							
n							
Day 5	10	10	10	10	10	10	10
Day 21	9	10	10	9	7	9	10
Week 14	10	10	10	9	10	10	10
Leukocytes (10^3/µL)							
Day 5	8.82 ± 0.59	9.42 ± 0.26	8.76 ± 0.62	8.91 ± 0.54	9.18 ± 0.66	9.46 ± 0.40	9.66 ± 0.36
Day 21	8.79 ± 0.36	8.72 ± 0.57	8.83 ± 0.38	9.10 ± 0.37	9.24 ± 0.43	7.91 ± 0.48	8.79 ± 0.33
Week 14	12.78 ± 0.31	12.77 ± 0.33	12.32 ± 0.40	11.93 ± 0.66	12.27 ± 0.43	11.89 ± 0.32	11.03 ± 0.66
Segmented neutrophils (10^3/µL)							
Day 5	1.37 ± 0.13	1.35 ± 0.10	1.38 ± 0.12	1.33 ± 0.16	1.48 ± 0.09	1.34 ± 0.08	1.84 ± 0.19
Day 21	1.37 ± 0.11	1.10 ± 0.12	1.23 ± 0.22	1.10 ± 0.11	1.55 ± 0.39	0.98 ± 0.15	1.01 ± 0.11
Week 14	1.66 ± 0.12	1.55 ± 0.14	1.80 ± 0.14	1.71 ± 0.26	2.05 ± 0.26	1.63 ± 0.18	1.47 ± 0.13
Bands (10^3/µL)							
Day 5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Metamyelocytes (10^3/µL)							
Day 5	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 21	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Myelocytes (10^3/µL)							
Day 5	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 21	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lymphocytes (10^3/µL)							
Day 5	7.28 ± 0.52	7.87 ± 0.20	7.23 ± 0.54	7.39 ± 0.48	7.62 ± 0.60	7.92 ± 0.38	7.71 ± 0.24
Day 21	7.29 ± 0.31	7.48 ± 0.52	7.48 ± 0.36	7.85 ± 0.31	7.59 ± 0.42	6.81 ± 0.39	7.66 ± 0.32
Week 14	10.84 ± 0.31	10.92 ± 0.39	10.17 ± 0.31	10.01 ± 0.48	9.94 ± 0.38	9.96 ± 0.32	9.36 ± 0.59
Atypical lymphocytes (10^3/µL)							
Day 5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10^3/µL)							
Day 5	0.18 ± 0.05	0.22 ± 0.06	0.18 ± 0.04	0.19 ± 0.03	0.11 ± 0.05	0.19 ± 0.04	0.15 ± 0.04
Day 21	0.15 ± 0.06	0.12 ± 0.04	0.11 ± 0.03	0.12 ± 0.03	0.13 ± 0.07	0.10 ± 0.04	0.11 ± 0.04
Week 14	0.16 ± 0.04	0.20 ± 0.07	0.27 ± 0.03	0.13 ± 0.05	0.16 ± 0.04	0.22 ± 0.05	0.10 ± 0.04
Basophils (10^3/µL)							
Day 5	0.009 ± 0.009	0.010 ± 0.010	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 21	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.010 ± 0.010	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)							
Day 5	0.01 ± 0.01	0.00 ± 0.00	0.04 ± 0.02	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.02	0.03 ± 0.02
Day 21	0.01 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.04	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Week 14	0.12 ± 0.04	0.10 ± 0.04	0.08 ± 0.03	0.08 ± 0.02	0.13 ± 0.04	0.09 ± 0.04	0.10 ± 0.04

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

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male (continued)							
Clinical Chemistry							
n							
Day 5	10	10	10	10	10	10	10
Day 21	9	10	10	9	7	9	10
Week 14	10	10	10	9	9	10	10
Urea nitrogen (mg/dL)							
Day 5	17.1 ± 0.5	17.0 ± 0.3	16.9 ± 0.3	16.1 ± 0.4	17.0 ± 0.6	16.2 ± 0.4	15.1 ± 0.5**
Creatinine (mg/dL)							
Day 5	0.57 ± 0.02	0.57 ± 0.02	0.54 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	0.56 ± 0.02
Day 21	0.67 ± 0.02	0.65 ± 0.02	0.67 ± 0.02	0.66 ± 0.02	0.66 ± 0.02	0.69 ± 0.02	0.67 ± 0.02
Week 14	0.69 ± 0.02	0.70 ± 0.00	0.70 ± 0.02	0.70 ± 0.02	0.69 ± 0.01	0.72 ± 0.01	0.71 ± 0.01
Total protein (g/dL)							
Day 5	6.0 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	6.1 ± 0.1	5.8 ± 0.1	5.9 ± 0.1
Day 21	6.6 ± 0.1	6.6 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.4 ± 0.1
Week 14	7.0 ± 0.0	7.1 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	7.1 ± 0.0	7.1 ± 0.1	7.2 ± 0.1
Albumin (g/dL)							
Day 5	4.3 ± 0.0	4.2 ± 0.0	4.1 ± 0.1	4.1 ± 0.1	4.3 ± 0.0	4.1 ± 0.0	4.2 ± 0.0
Day 21	4.8 ± 0.1	4.8 ± 0.1	4.6 ± 0.1	4.7 ± 0.0	4.7 ± 0.0	4.8 ± 0.0	4.6 ± 0.0*
Week 14	4.9 ± 0.0	4.9 ± 0.0	5.0 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	5.0 ± 0.0	5.0 ± 0.1
Cholesterol (mg/dL)							
Day 5	74 ± 2	72 ± 2	72 ± 2	72 ± 2	76 ± 2	74 ± 2	68 ± 2
Day 21	75 ± 2	68 ± 2	68 ± 2	69 ± 2	69 ± 2	67 ± 2	66 ± 1
Week 14	72 ± 2	77 ± 1	77 ± 2	73 ± 1	74 ± 1	74 ± 1	76 ± 3
Alanine aminotransferase (IU/L)							
Day 5	39 ± 1	37 ± 2	37 ± 1	40 ± 1	37 ± 1	37 ± 3	34 ± 2
Day 21	43 ± 1	41 ± 2	40 ± 1	39 ± 2	38 ± 0	37 ± 2	36 ± 3**
Week 14	55 ± 2	56 ± 2	55 ± 2	59 ± 3	53 ± 3	51 ± 3	64 ± 2
Alkaline phosphatase (IU/L)							
Day 5	652 ± 15	629 ± 12	647 ± 12	644 ± 11	620 ± 13	635 ± 17	645 ± 12
Day 21	499 ± 11	495 ± 5	483 ± 10	497 ± 8	472 ± 13	482 ± 11	464 ± 12
Week 14	236 ± 4	231 ± 6	231 ± 4	224 ± 4	222 ± 6	219 ± 6	225 ± 6
Creatine kinase (IU/L)							
Day 21	219 ± 34	343 ± 83	246 ± 29	262 ± 42	290 ± 102	269 ± 48	246 ± 19
Week 14	155 ± 19	155 ± 24	158 ± 14	181 ± 29	151 ± 13	171 ± 27	203 ± 21
Sorbitol dehydrogenase (IU/L)							
Day 5	12 ± 1	13 ± 1	14 ± 1	12 ± 1	11 ± 0	13 ± 0	11 ± 1
Day 21	15 ± 1	15 ± 1	17 ± 1	17 ± 1	14 ± 1	18 ± 1	16 ± 1
Week 14	11 ± 1	13 ± 1	13 ± 1	14 ± 2	12 ± 2	13 ± 2	14 ± 1
5′-Nucleotidase (IU/L)							
Day 5	27 ± 1	26 ± 1	26 ± 1	26 ± 0	26 ± 0	25 ± 1	25 ± 0
Day 21	28 ± 1	27 ± 1	26 ± 0	27 ± 0	26 ± 1	26 ± 0	25 ± 0**
Week 14	30 ± 1	30 ± 1	29 ± 1	29 ± 0	29 ± 0	28 ± 0*	28 ± 1*
Bile acids (μmol/L)							
Day 5	42.3 ± 5.4	38.6 ± 4.1	43.8 ± 4.8	48.4 ± 4.5	40.3 ± 3.6	53.8 ± 3.8	36.3 ± 3.6
Day 21	30.4 ± 3.1	35.9 ± 4.1	34.0 ± 4.3	37.0 ± 4.5	29.5 ± 4.3	40.4 ± 4.4	26.1 ± 2.8
Week 14	18.6 ± 2.3	27.7 ± 4.4	34.0 ± 5.3	26.6 ± 3.8	39.2 ± 7.1	22.9 ± 3.4	31.2 ± 6.6

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

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Female							
n	10	10	10	10	10	10	10
Hematology							
Automated hematocrit (%)							
Day 5	42.1 ± 0.7	41.2 ± 0.6	41.1 ± 0.6	40.3 ± 0.6	41.2 ± 0.5	41.0 ± 0.4	40.9 ± 0.5
Day 21	45.4 ± 0.4	45.4 ± 0.4	45.2 ± 0.3	44.0 ± 0.3	44.6 ± 0.3	43.7 ± 0.5	43.9 ± 0.8
Week 14	40.9 ± 0.4	41.9 ± 0.3	41.5 ± 0.5	41.2 ± 0.5	41.2 ± 0.2	40.4 ± 0.3**	40.1 ± 0.4**
Manual hematocrit (%)							
Day 5	45.3 ± 0.6	44.8 ± 0.6	45.1 ± 0.6	43.7 ± 0.7	44.6 ± 0.6	44.3 ± 0.4	44.7 ± 0.5
Day 21	48.7 ± 0.4	49.2 ± 0.5	49.0 ± 0.4	47.5 ± 0.4	48.3 ± 0.4	47.6 ± 0.5	48.0 ± 0.6
Week 14	44.2 ± 0.5	44.9 ± 0.3	44.6 ± 0.4	44.1 ± 0.4	44.3 ± 0.2	43.6 ± 0.3**	43.3 ± 0.4**
Hemoglobin (g/dL)							
Day 5	15.5 ± 0.2	15.1 ± 0.2	15.3 ± 0.2	14.9 ± 0.2	15.2 ± 0.2	15.1 ± 0.1	15.1 ± 0.2
Day 21	16.6 ± 0.1	16.6 ± 0.1	16.6 ± 0.2	16.1 ± 0.2*	16.4 ± 0.1	15.9 ± 0.2**	16.1 ± 0.2*
Week 14	15.0 ± 0.1	15.2 ± 0.1	15.2 ± 0.2	15.0 ± 0.1	15.0 ± 0.1	14.7 ± 0.1**	14.5 ± 0.1**
Erythrocytes (10^6/µL)							
Day 5	7.14 ± 0.13	7.00 ± 0.13	7.00 ± 0.13	6.83 ± 0.13	6.95 ± 0.10	6.92 ± 0.09	6.92 ± 0.10
Day 21	7.46 ± 0.07	7.52 ± 0.08	7.48 ± 0.06	7.28 ± 0.07*	7.34 ± 0.06*	7.21 ± 0.08**	7.21 ± 0.14*
Week 14	7.41 ± 0.07	7.59 ± 0.06	7.58 ± 0.10	7.50 ± 0.08	7.49 ± 0.04	7.34 ± 0.05**	7.20 ± 0.08**
Reticulocytes (10^6/µL)							
Day 5	0.17 ± 0.02	0.19 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
Day 21	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.00	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Week 14	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.08 ± 0.01
Nucleated erythrocytes (10^3/µL)							
Day 5	1.10 ± 0.31	0.50 ± 0.34	0.70 ± 0.30	0.50 ± 0.31	0.20 ± 0.20	0.80 ± 0.29	0.80 ± 0.29
Day 21	0.10 ± 0.10	0.00 ± 0.00	0.30 ± 0.15	0.20 ± 0.20	0.20 ± 0.13	0.00 ± 0.00	0.20 ± 0.20
Week 14	0.40 ± 0.22	0.60 ± 0.31	0.60 ± 0.16	0.70 ± 0.30	0.60 ± 0.27	0.20 ± 0.13	0.40 ± 0.22
Mean cell volume (fL)							
Day 5	59.0 ± 0.2	58.9 ± 0.3	58.8 ± 0.3	59.0 ± 0.3	59.2 ± 0.3	59.3 ± 0.2	59.1 ± 0.3
Day 21	60.8 ± 0.2	60.4 ± 0.2	60.4 ± 0.2	60.4 ± 0.2	60.8 ± 0.2	60.7 ± 0.2	60.9 ± 0.2
Week 14	55.2 ± 0.1	55.1 ± 0.1	54.8 ± 0.1	54.9 ± 0.1	55.0 ± 0.2	55.1 ± 0.1	55.7 ± 0.0**
Mean cell hemoglobin (pg)							
Day 5	21.7 ± 0.2	21.6 ± 0.1	21.8 ± 0.1	21.9 ± 0.1	21.9 ± 0.1	21.8 ± 0.1	21.8 ± 0.1
Day 21	22.3 ± 0.2	22.0 ± 0.2	22.2 ± 0.1	22.1 ± 0.1	22.3 ± 0.1	22.1 ± 0.1	22.3 ± 0.2
Week 14	20.2 ± 0.1	20.1 ± 0.0	20.1 ± 0.1	20.0 ± 0.1[b]	20.0 ± 0.1	20.0 ± 0.1	20.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)							
Day 5	36.7 ± 0.2	36.7 ± 0.2	37.1 ± 0.2	37.1 ± 0.2	37.0 ± 0.1	36.8 ± 0.2	37.0 ± 0.2
Day 21	36.6 ± 0.2	36.5 ± 0.2	36.8 ± 0.2	36.6 ± 0.2	36.7 ± 0.2	36.5 ± 0.2	36.7 ± 0.2
Week 14	36.7 ± 0.1	36.4 ± 0.1	36.7 ± 0.1	36.5 ± 0.2	36.4 ± 0.1	36.3 ± 0.2	36.2 ± 0.2
Platelets (10^3/µL)							
Day 5	853.4 ± 14.5	878.6 ± 12.2	854.0 ± 23.1	841.3 ± 18.1	828.5 ± 23.2	856.3 ± 21.2	844.2 ± 19.7
Day 21	804.9 ± 12.3	787.2 ± 20.2	772.8 ± 22.5	748.4 ± 22.0	779.7 ± 10.7	778.7 ± 19.7	769.0 ± 19.7
Week 14	662.0 ± 14.6	664.5 ± 11.5	655.0 ± 13.9	664.4 ± 11.8	681.6 ± 26.7	646.4 ± 9.5	663.9 ± 9.9

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

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Female (continued)							
n	10	10	10	10	10	10	10
Hematology (continued)							
Leukocytes (10^3/μL)							
Day 5	9.43 ± 0.39	9.75 ± 0.42	9.20 ± 0.33	9.06 ± 0.32	9.35 ± 0.25	9.89 ± 0.51	9.22 ± 0.46
Day 21	9.64 ± 0.37	9.53 ± 0.40	9.49 ± 0.42	9.31 ± 0.40	8.87 ± 0.51	8.87 ± 0.36	8.90 ± 0.26
Week 14	10.28 ± 0.38	10.21 ± 0.69	10.01 ± 0.33	9.85 ± 0.46	8.98 ± 0.36	9.32 ± 0.59	9.73 ± 0.49
Segmented neutrophils (10^3/μL)							
Day 5	1.11 ± 0.11	1.05 ± 0.17	1.21 ± 0.13	1.29 ± 0.17	1.05 ± 0.13	1.13 ± 0.26	1.08 ± 0.14
Day 21	1.09 ± 0.10	1.36 ± 0.22	1.35 ± 0.19	1.31 ± 0.20	1.09 ± 0.17	1.04 ± 0.15	0.93 ± 0.10
Week 14	1.60 ± 0.16	1.97 ± 0.50	1.55 ± 0.16	1.63 ± 0.17	1.49 ± 0.16	1.25 ± 0.11	1.74 ± 0.21
Bands (10^3/μL)							
Day 5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Metamyelocytes (10^3/μL)							
Day 5	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 21	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Myelocytes (10^3/μL)							
Day 5	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 21	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lymphocytes (10^3/μL)							
Day 5	8.27 ± 0.38	8.54 ± 0.37	7.91 ± 0.36	7.64 ± 0.25	8.04 ± 0.26	8.57 ± 0.42	8.01 ± 0.43
Day 21	8.42 ± 0.34	8.05 ± 0.33	8.03 ± 0.35	7.90 ± 0.37	7.69 ± 0.38	7.72 ± 0.22	7.77 ± 0.29
Week 14	8.52 ± 0.35	8.04 ± 0.29	8.19 ± 0.32	8.07 ± 0.33	7.28 ± 0.34	7.92 ± 0.53	7.79 ± 0.43
Atypical lymphocytes (10^3/μL)							
Day 5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10^3/μL)							
Day 5	0.07 ± 0.02	0.17 ± 0.04	0.06 ± 0.02	0.15 ± 0.04	0.24 ± 0.04	0.17 ± 0.04	0.13 ± 0.06
Day 21	0.10 ± 0.03	0.09 ± 0.03	0.06 ± 0.02	0.07 ± 0.02	0.05 ± 0.02	0.08 ± 0.04	0.13 ± 0.04
Week 14	0.08 ± 0.03	0.14 ± 0.04	0.20 ± 0.06	0.10 ± 0.02	0.11 ± 0.04	0.11 ± 0.04	0.15 ± 0.05
Basophils (10^3/μL)							
Day 5	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 21	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)							
Day 5	0.01 ± 0.01	0.02 ± 0.02	0.05 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.04 ± 0.02
Day 21	0.04 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.08 ± 0.03
Week 14	0.09 ± 0.03	0.07 ± 0.03	0.08 ± 0.02	0.06 ± 0.03	0.10 ± 0.03	0.05 ± 0.02	0.04 ± 0.02

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

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Female (continued)							
n	10	10	10	10	10	10	10
Clinical Chemistry							
Urea nitrogen (mg/dL)							
Day 5	21.1 ± 0.5	19.1 ± 0.7	20.1 ± 0.7	18.7 ± 1.1	18.6 ± 0.6	18.9 ± 0.7	19.0 ± 0.7
Creatinine (mg/dL)							
Day 5	0.60 ± 0.02	0.60 ± 0.00	0.59 ± 0.01	0.62 ± 0.03	0.60 ± 0.02	0.60 ± 0.00	0.60 ± 0.02
Day 21	0.65 ± 0.02	0.67 ± 0.02	0.64 ± 0.02	0.67 ± 0.02	0.68 ± 0.01	0.66 ± 0.02	0.67 ± 0.03
Week 14	0.71 ± 0.01	0.72 ± 0.02	0.71 ± 0.01	0.72 ± 0.01	0.71 ± 0.02	0.70 ± 0.02	0.70 ± 0.02
Total protein (g/dL)							
Day 5	5.8 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.7 ± 0.1[b]	5.7 ± 0.1	5.8 ± 0.1
Day 21	6.2 ± 0.1	6.1 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	6.1 ± 0.1
Week 14	7.0 ± 0.1	7.2 ± 0.1	6.9 ± 0.1	7.1 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.1**
Albumin (g/dL)							
Day 5	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.3 ± 0.0	4.3 ± 0.0
Day 21	4.7 ± 0.0	4.8 ± 0.1	4.8 ± 0.0	4.7 ± 0.1	4.8 ± 0.0	4.6 ± 0.1	4.7 ± 0.1
Week 14	5.0 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	5.0 ± 0.0	4.9 ± 0.1	4.9 ± 0.1	4.7 ± 0.0*
Cholesterol (mg/dL)							
Day 5	98 ± 3	95 ± 3	100 ± 3	93 ± 3	96 ± 2	96 ± 2	96 ± 3
Day 21	94 ± 2	92 ± 2	95 ± 3	96 ± 3	94 ± 2	97 ± 2	97 ± 2
Week 14	94 ± 3	92 ± 2	95 ± 3	101 ± 2	97 ± 4	94 ± 2	94 ± 3
Alanine aminotransferase (IU/L)							
Day 5	37 ± 1	33 ± 1	34 ± 1	31 ± 1	33 ± 1	33 ± 1	33 ± 2
Day 21	34 ± 1	34 ± 2	34 ± 2	33 ± 1	33 ± 2	33 ± 1	34 ± 2
Week 14	42 ± 2	46 ± 4	44 ± 3	36 ± 2	34 ± 2*	38 ± 3	42 ± 2
Alkaline phosphatase (IU/L)							
Day 5	530 ± 11	502 ± 7	504 ± 6	494 ± 8	493 ± 7	502 ± 11	476 ± 11
Day 21	404 ± 10	383 ± 8	377 ± 5	367 ± 8	352 ± 7**	356 ± 6*	353 ± 6**
Week 14	191 ± 5	191 ± 10	198 ± 6	203 ± 5	206 ± 4	187 ± 6	176 ± 3
Creatine kinase (IU/L)							
Day 21	273 ± 50	257 ± 33[b]	358 ± 61	248 ± 31	378 ± 85	292 ± 52	274 ± 48
Week 14	217 ± 20	172 ± 15	218 ± 22	201 ± 17	181 ± 19	196 ± 17	186 ± 11
Sorbitol dehydrogenase (IU/L)							
Day 5	6 ± 0	6 ± 1	5 ± 0	5 ± 0	5 ± 0	5 ± 1	6 ± 1
Day 21	20 ± 1	21 ± 1	24 ± 1	20 ± 1	19 ± 1	21 ± 1	20 ± 2
Week 14	18 ± 1	19 ± 2	19 ± 1	17 ± 1	17 ± 1	17 ± 1	18 ± 1
5′-Nucleotidase (IU/L)							
Day 5	34 ± 1	33 ± 1	36 ± 1	33 ± 1	34 ± 1	32 ± 0	32 ± 1
Day 21	35 ± 1	37 ± 1	37 ± 1	35 ± 1	36 ± 1	34 ± 1	33 ± 1**
Week 14	32 ± 1	34 ± 1	31 ± 1*	32 ± 1*	34 ± 1	32 ± 1	29 ± 1**
Bile acids (μmol/L)							
Day 5	27.9 ± 3.5	26.8 ± 2.5	22.6 ± 2.1	28.0 ± 4.7	29.2 ± 2.4	33.6 ± 4.0	21.9 ± 3.0
Day 21	32.2 ± 3.4	27.1 ± 3.5	27.6 ± 2.7	28.5 ± 2.9	35.6 ± 2.9	33.5 ± 3.4	32.1 ± 4.4
Week 14	42.1 ± 4.4	59.2 ± 9.5	64.3 ± 9.5	52.3 ± 6.4	58.7 ± 7.9	55.4 ± 6.1	59.8 ± 6.6

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

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

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
n	10	10	10	10	10	10	10
Male							
Creatinine (mg/dL)	0.40 ± 0.02	0.40 ± 0.00	0.40 ± 0.02	0.41 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.40 ± 0.00
Total protein (g/dL)	5.7 ± 0.1	5.6 ± 0.1	5.6 ± 0.0	5.6 ± 0.0	5.6 ± 0.1	5.5 ± 0.1	5.5 ± 0.0
Albumin (g/dL)	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.0 ± 0.1	4.1 ± 0.1
Cholesterol (mg/dL)	141 ± 7	135 ± 7	139 ± 3	135 ± 3	140 ± 7	128 ± 4	128 ± 3
Alanine aminotransferase (IU/L)	47 ± 8	35 ± 9	34 ± 9	40 ± 7	46 ± 6	39 ± 7	35 ± 7
Alkaline phosphatase (IU/L)	86 ± 3	83 ± 3	88 ± 2	86 ± 2	86 ± 2	90 ± 3	91 ± 2
Creatine kinase (IU/L)	750 ± 228	580 ± 131	653 ± 216	631 ± 174	389 ± 96	269 ± 32	616 ± 156
Sorbitol dehydrogenase (IU/L)	46 ± 1	45 ± 2	47 ± 2	52 ± 1*	51 ± 3	45 ± 1	51 ± 2
5′-Nucleotidase (IU/L)	18 ± 0	17 ± 1	17 ± 0	17 ± 0	16 ± 0	15 ± 0*	15 ± 0*
Bile acids (µmol/L)	15.6 ± 0.6	16.6 ± 0.8	16.7 ± 0.7	16.6 ± 0.8	17.5 ± 0.8	16.1 ± 0.7	16.0 ± 1.0
Female							
Creatinine (mg/dL)	0.46 ± 0.02	0.45 ± 0.02	0.49 ± 0.01	0.48 ± 0.01	0.46 ± 0.02	0.45 ± 0.02	0.46 ± 0.02
Total protein (g/dL)	5.5 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.5 ± 0.0	5.8 ± 0.0	5.7 ± 0.1	5.7 ± 0.1
Albumin (g/dL)	4.5 ± 0.0	4.5 ± 0.1	4.4 ± 0.0	4.4 ± 0.0	4.6 ± 0.0	4.6 ± 0.0	4.6 ± 0.1
Cholesterol (mg/dL)	112 ± 3	110 ± 4	115 ± 2	112 ± 3	112 ± 2	114 ± 2	115 ± 3
Alanine aminotransferase (IU/L)	29 ± 2	28 ± 3	37 ± 3	55 ± 22	57 ± 14*	45 ± 12	50 ± 14
Alkaline phosphatase (IU/L)	132 ± 4	142 ± 5	143 ± 8	124 ± 6	149 ± 3	137 ± 4	144 ± 6
Creatine kinase (IU/L)	356 ± 56	330 ± 55	372 ± 27	508 ± 73	252 ± 39	295 ± 34	455 ± 86
Sorbitol dehydrogenase (IU/L)	41 ± 1	39 ± 1	42 ± 2	45 ± 2*	44 ± 3[b]	43 ± 2	44 ± 2
5′-Nucleotidase (IU/L)	53 ± 2	53 ± 2	56 ± 2	55 ± 4	56 ± 2	56 ± 2	54 ± 2
Bile acids (µmol/L)	14.3 ± 0.4	14.8 ± 0.5	17.5 ± 0.6*	15.6 ± 0.7	18.3 ± 2.2	15.1 ± 0.7	16.6 ± 0.7

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

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 14-Week Feed Study of *trans*-1,2-Dichloroethylene[a]

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
n	10	10	10	10	10	10	10
Male							
Necropsy body wt	362 ± 7	360 ± 6	365 ± 5	361 ± 3	357 ± 5	350 ± 6	339 ± 4**
Heart							
Absolute	1.056 ± 0.022	1.019 ± 0.018	1.010 ± 0.014	1.024 ± 0.017	0.997 ± 0.021	0.966 ± 0.019	0.968 ± 0.017
Relative	0.292 ± 0.004	0.283 ± 0.004	0.277 ± 0.003	0.284 ± 0.003	0.279 ± 0.005	0.277 ± 0.005	0.285 ± 0.004
R. Kidney							
Absolute	1.193 ± 0.028	1.258 ± 0.029	1.254 ± 0.034	1.244 ± 0.026	1.234 ± 0.016	1.167 ± 0.029*	1.172 ± 0.023*
Relative	0.330 ± 0.004	0.350 ± 0.006	0.344 ± 0.006	0.345 ± 0.005	0.345 ± 0.002	0.333 ± 0.004	0.346 ± 0.007
Liver							
Absolute	12.376 ± 0.264	12.488 ± 0.389	12.901 ± 0.230	13.210 ± 0.405	12.605 ± 0.314	12.221 ± 0.310	12.326 ± 0.219
Relative	3.427 ± 0.063	3.465 ± 0.058	3.538 ± 0.032	3.658 ± 0.099	3.524 ± 0.050	3.492 ± 0.048	3.634 ± 0.056
Lung							
Absolute	1.612 ± 0.047	1.571 ± 0.036	1.719 ± 0.057	1.659 ± 0.087	1.560 ± 0.036	1.467 ± 0.034	1.466 ± 0.031
Relative	0.447 ± 0.012	0.437 ± 0.010	0.471 ± 0.011	0.459 ± 0.022	0.437 ± 0.009	0.420 ± 0.011	0.432 ± 0.009
R. Testis							
Absolute	1.452 ± 0.031	1.440 ± 0.034	1.477 ± 0.030	1.361 ± 0.063	1.576 ± 0.104	1.494 ± 0.026	1.460 ± 0.014
Relative	0.402 ± 0.006	0.401 ± 0.011	0.405 ± 0.007	0.378 ± 0.018	0.441 ± 0.027	0.428 ± 0.007	0.431 ± 0.006
Thymus							
Absolute	0.362 ± 0.016	0.346 ± 0.010	0.381 ± 0.013	0.363 ± 0.015	0.349 ± 0.013	0.350 ± 0.014	0.331 ± 0.018
Relative	0.100 ± 0.004	0.097 ± 0.004	0.104 ± 0.003	0.100 ± 0.004	0.098 ± 0.004	0.100 ± 0.004	0.097 ± 0.005
Female							
Necropsy body wt	196 ± 3	190 ± 4	198 ± 3	203 ± 2*	198 ± 3	196 ± 3	191 ± 2
Heart							
Absolute	0.651 ± 0.015	0.644 ± 0.014	0.643 ± 0.013	0.658 ± 0.009	0.638 ± 0.011	0.640 ± 0.013	0.648 ± 0.012
Relative	0.332 ± 0.005	0.339 ± 0.005	0.325 ± 0.005	0.324 ± 0.005	0.322 ± 0.004	0.327 ± 0.007	0.338 ± 0.004
R. Kidney							
Absolute	0.672 ± 0.013	0.662 ± 0.010	0.662 ± 0.009	0.707 ± 0.009	0.666 ± 0.015	0.657 ± 0.016	0.683 ± 0.016
Relative	0.343 ± 0.005	0.349 ± 0.006	0.335 ± 0.003	0.349 ± 0.004	0.336 ± 0.004	0.335 ± 0.004	0.356 ± 0.005
Liver							
Absolute	5.895 ± 0.148	5.579 ± 0.130	6.011 ± 0.126	6.536 ± 0.145**	6.133 ± 0.108*	6.137 ± 0.146*	6.160 ± 0.139*
Relative	3.005 ± 0.052	2.937 ± 0.038	3.040 ± 0.052	3.220 ± 0.066**	3.100 ± 0.051**	3.132 ± 0.052**	3.216 ± 0.051**
Lung							
Absolute	1.104 ± 0.029	1.090 ± 0.028	1.056 ± 0.040	1.064 ± 0.019	1.051 ± 0.030	1.099 ± 0.033	1.062 ± 0.021
Relative	0.563 ± 0.011	0.574 ± 0.011	0.534 ± 0.020	0.524 ± 0.009*	0.530 ± 0.009	0.561 ± 0.014	0.555 ± 0.008
Thymus							
Absolute	0.275 ± 0.012	0.253 ± 0.012	0.269 ± 0.006	0.283 ± 0.006	0.287 ± 0.008*	0.266 ± 0.009	0.268 ± 0.008
Relative	0.140 ± 0.006	0.133 ± 0.004	0.136 ± 0.003	0.140 ± 0.002	0.145 ± 0.004	0.136 ± 0.005	0.140 ± 0.004

* 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 g organ weight/g body weight as a percentage (mean ± standard error).

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

	Untreated Control	Vehicle Control	3,125 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
n	10	10	10	10	10	10	10
Male							
Necropsy body wt	30.6 ± 0.9	30.3 ± 0.6	29.8 ± 0.4	30.5 ± 0.6	29.2 ± 1.0	29.0 ± 0.5	28.2 ± 0.5*
Heart							
Absolute	0.145 ± 0.003	0.146 ± 0.003	0.144 ± 0.002	0.144 ± 0.005	0.140 ± 0.003	0.147 ± 0.003	0.138 ± 0.002
Relative	0.473 ± 0.009	0.484 ± 0.008	0.482 ± 0.007	0.473 ± 0.011	0.482 ± 0.008	0.505 ± 0.008	0.489 ± 0.004
R. Kidney							
Absolute	0.273 ± 0.009	0.275 ± 0.006	0.287 ± 0.009	0.291 ± 0.009	0.263 ± 0.011	0.270 ± 0.005	0.261 ± 0.005
Relative	0.894 ± 0.028	0.908 ± 0.012	0.963 ± 0.024	0.953 ± 0.017	0.902 ± 0.032	0.933 ± 0.016	0.928 ± 0.010
Liver							
Absolute	1.397 ± 0.054	1.317 ± 0.027	1.357 ± 0.039	1.403 ± 0.043	1.380 ± 0.040	1.371 ± 0.023	1.401 ± 0.033
Relative	4.554 ± 0.076	4.347 ± 0.056	4.552 ± 0.113	4.597 ± 0.115	4.745 ± 0.084**	4.736 ± 0.079**	4.979 ± 0.111**
Lung							
Absolute	0.195 ± 0.008	0.197 ± 0.012	0.193 ± 0.006	0.204 ± 0.009	0.187 ± 0.004	0.201 ± 0.010	0.201 ± 0.006
Relative	0.641 ± 0.030	0.647 ± 0.033	0.648 ± 0.018	0.670 ± 0.030	0.648 ± 0.021	0.699 ± 0.045	0.715 ± 0.018
R. Testis							
Absolute	0.120 ± 0.002	0.120 ± 0.004	0.121 ± 0.007	0.117 ± 0.006	0.117 ± 0.003	0.123 ± 0.003	0.124 ± 0.002
Relative	0.396 ± 0.010	0.397 ± 0.014	0.407 ± 0.022	0.387 ± 0.022	0.405 ± 0.011	0.425 ± 0.010	0.439 ± 0.007
Thymus							
Absolute	0.042 ± 0.003	0.039 ± 0.002	0.038 ± 0.002	0.039 ± 0.003	0.035 ± 0.002	0.040 ± 0.002	0.036 ± 0.002
Relative	0.139 ± 0.009	0.128 ± 0.005	0.126 ± 0.006	0.126 ± 0.009	0.119 ± 0.005	0.140 ± 0.008	0.127 ± 0.006
Female							
Necropsy body wt	24.1 ± 0.5	23.3 ± 0.3	23.8 ± 0.5	25.0 ± 0.6	22.0 ± 0.4	22.4 ± 0.5	21.6 ± 0.4*
Heart							
Absolute	0.125 ± 0.003	0.120 ± 0.005	0.119 ± 0.002	0.130 ± 0.003	0.117 ± 0.002	0.112 ± 0.002	0.113 ± 0.001
Relative	0.518 ± 0.009	0.517 ± 0.018	0.499 ± 0.006	0.520 ± 0.013	0.530 ± 0.005	0.502 ± 0.010	0.527 ± 0.011
R. Kidney							
Absolute	0.183 ± 0.005	0.175 ± 0.004	0.172 ± 0.005	0.187 ± 0.005	0.167 ± 0.003	0.164 ± 0.004	0.162 ± 0.004*
Relative	0.760 ± 0.012	0.754 ± 0.018	0.723 ± 0.009	0.747 ± 0.010	0.760 ± 0.011	0.732 ± 0.016	0.750 ± 0.015
Liver							
Absolute	1.062 ± 0.109	1.076 ± 0.025	1.128 ± 0.035	1.244 ± 0.054**	1.058 ± 0.019	1.141 ± 0.028	1.107 ± 0.028
Relative	4.370 ± 0.429	4.621 ± 0.070	4.738 ± 0.068	4.970 ± 0.127	4.813 ± 0.050	5.115 ± 0.139**	5.117 ± 0.080**
Lung							
Absolute	0.199 ± 0.014	0.181 ± 0.010	0.180 ± 0.004	0.189 ± 0.007	0.170 ± 0.004	0.169 ± 0.004	0.172 ± 0.005
Relative	0.832 ± 0.066	0.773 ± 0.034	0.757 ± 0.015	0.758 ± 0.027	0.772 ± 0.012	0.758 ± 0.022	0.798 ± 0.027
Thymus							
Absolute	0.042 ± 0.003	0.040 ± 0.002	0.051 ± 0.004**	0.048 ± 0.001	0.041 ± 0.002	0.042 ± 0.002	0.043 ± 0.002
Relative	0.173 ± 0.012	0.172 ± 0.009	0.215 ± 0.014*	0.193 ± 0.004	0.186 ± 0.009	0.187 ± 0.014	0.199 ± 0.008

* 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 g organ weight/g body weight as a percentage (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 *trans*-1,2-Dichloroethylene[a]

	Untreated Control	Vehicle Control	12,500 ppm	25,000 ppm	50,000 ppm
n	10	10	10	10	10
Weights (g)					
Necropsy body wt	361 ± 7	360 ± 6	357 ± 5	350 ± 6	339 ± 4*
L. Cauda epididymis	0.1677 ± 0.0058	0.1675 ± 0.0054	0.1719 ± 0.0053	0.1673 ± 0.0025	0.1598 ± 0.0034
L. Epididymis	0.4619 ± 0.0089	0.4737 ± 0.0126	0.4696 ± 0.0095	0.4701 ± 0.0093	0.4554 ± 0.0051
L. Testis	1.5170 ± 0.0244	1.4782 ± 0.0223	1.5200 ± 0.0244[b]	1.5303 ± 0.0218	1.5039 ± 0.0165
Spermatid measurements					
Spermatid heads (10^7/g testis)	10.413 ± 0.421	10.535 ± 0.247	9.913 ± 0.194[b]	9.735 ± 0.255*	10.258 ± 0.219
Spermatid heads (10^7/testis)	15.745 ± 0.538	15.540 ± 0.267	15.180 ± 0.259	14.885 ± 0.401	15.420 ± 0.330
Spermatid count (mean/10^{-4} mL suspension)	78.725 ± 2.689	77.700 ± 1.336	75.900 ± 1.294	74.425 ± 2.007	77.100 ± 1.650
Epididymal spermatozoal measurements					
Motility (%)	87.60 ± 0.56	87.82 ± 0.54	87.46 ± 0.48	87.69 ± 0.37	86.71 ± 0.48
Concentration (10^6/g cauda epididymal tissue)	382 ± 24	364 ± 43	405 ± 17	410 ± 11	387 ± 24

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test (body weight) or Dunn's test (spermatid heads per g testis)
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid heads per testis, spermatid count, and epididymal spermatozoal measurements).
[b] n=9

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

	Untreated Control	Vehicle Control	12,500 ppm	25,000 ppm	50,000 ppm
n	10	10	10	10	10
Necropsy body wt (g)	196 ± 3	190 ± 4	198 ± 3	196 ± 3	191 ± 2
Estrous cycle length (days)	5.000 ± 0.129	5.000 ± 0.000	5.000 ± 0.075	5.150 ± 0.150	5.667 ± 0.373[b]
Estrous stages (% of cycle)					
Diestrus	39.2	40.0	41.7	40.0	46.7
Proestrus	18.3	15.8	20.0	19.2	15.8
Estrus	25.0	24.2	20.0	21.7	20.0
Metestrus	17.5	20.0	18.3	19.2	17.5

[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). 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 1 of 10 animals.

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

	Untreated Control	Vehicle Control	12,500 ppm	25,000 ppm	50,000 ppm
n	10	10	10	10	10
Weights (g)					
Necropsy body wt	30.6 ± 0.8	30.3 ± 0.6	29.2 ± 1.0	29.0 ± 0.5	28.2 ± 0.5
L. Cauda epididymis	0.0214 ± 0.0018	0.0223 ± 0.0010	0.0201 ± 0.0018	0.0215 ± 0.0018	0.0206 ± 0.0017
L. Epididymis	0.0515 ± 0.0023	0.0515 ± 0.0017	0.0521 ± 0.0037	0.0500 ± 0.0030	0.0507 ± 0.0028
L. Testis	0.1175 ± 0.0026	0.1184 ± 0.0041	0.1133 ± 0.0025	0.1206 ± 0.0029	0.1207 ± 0.0033
Spermatid measurements					
Spermatid heads (10^7/g testis)	22.614 ± 0.591	21.614 ± 0.528	23.267 ± 0.513	21.886 ± 0.443	22.289 ± 0.686
Spermatid heads (10^7/testis)	2.645 ± 0.036	2.546 ± 0.072	2.631 ± 0.068	2.634 ± 0.063	2.674 ± 0.050
Spermatid count (mean/10^{-4} mL suspension)	82.675 ± 1.083	79.575 ± 2.238	82.250 ± 2.092	82.325 ± 1.981	83.550 ± 1.550
Epididymal spermatozoal measurements					
Motility (%)	86.81 ± 1.05	85.99 ± 1.14	85.28 ± 0.86	76.12 ± 8.49	86.35 ± 1.07
Concentration (10^6/g cauda epididymal tissue)	593 ± 83	546 ± 77	395 ± 62	549 ± 108	574 ± 59

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

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

	Untreated Control	Vehicle Control	12,500 ppm	25,000 ppm	50,000 ppm
n	10	10	10	10	10
Necropsy body wt (g)	24.5 ± 0.3	23.3 ± 0.3	22.0 ± 0.4*	22.1 ± 0.5*	21.6 ± 0.4**
Estrous cycle length (days)	4.450 ± 0.398	4.000 ± 0.144[b]	4.800 ± 0.490	4.500 ± 0.565[b]	4.120 ± 0.088
Estrous stages (% of cycle)					
Diestrus	35.0	37.5	32.5	40.0	25.0
Proestrus	14.2	18.3	12.5	10.8	13.3
Estrus	30.0	28.3	35.0	29.2	37.5
Metestrus	20.8	15.8	20.0	20.0	24.2

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed 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 1 of 10 animals.

APPENDIX E
GENETIC TOXICOLOGY

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

Strain	Dose (µg/plate)	Revertants/Plate[b] −S9 Trial 1	−S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
Study performed at SRI International							
TA100	0	110 ± 7.5	130 ± 2.3	101 ± 2.2	121 ± 4.4	95 ± 2.3	127 ± 2.3
	100	100 ± 5.6	125 ± 13.0	128 ± 3.3	132 ± 18.4	88 ± 5.0	101 ± 7.0
	333	87 ± 3.2	114 ± 8.4	115 ± 5.7	125 ± 6.6	89 ± 1.3	103 ± 6.8
	1,000	91 ± 3.5	118 ± 11.9	106 ± 12.9	114 ± 14.5	92 ± 8.4	89 ± 2.7
	3,333	97 ± 6.7	116 ± 9.9	95 ± 7.0	125 ± 9.3	102 ± 12.4	84 ± 6.2
	6,666	93 ± 3.7			104 ± 13.7		
	10,000		Toxic	82 ± 4.5[c]		84 ± 5.8	111 ± 7.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		563 ± 7.9	652 ± 20.9	548 ± 14.0	664 ± 23.0	426 ± 13.9	287 ± 5.4
TA1535	0	18 ± 1.5	34 ± 1.9	12 ± 2.0	15 ± 3.6	11 ± 0.6	27 ± 0.9
	100	17 ± 1.9	37 ± 4.6	10 ± 1.2	14 ± 2.7	11 ± 1.5	21 ± 5.0
	333	16 ± 0.9	43 ± 3.0	6 ± 1.2	15 ± 1.5	7 ± 1.0	20 ± 2.6
	1,000	19 ± 1.5	43 ± 1.0	9 ± 1.5	18 ± 2.3	8 ± 0.9	17 ± 2.3
	3,333	15 ± 1.8	38 ± 5.6	7 ± 0.3	14 ± 1.7	7 ± 0.7	18 ± 1.9
	6,666	15 ± 1.3			17 ± 1.8		
	10,000		4 ± 4.0[c]	5 ± 0.3[c]		10 ± 0.9	12 ± 2.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		378 ± 11.1	588 ± 11.7	270 ± 21.7	484 ± 9.1	165 ± 8.4	91 ± 9.4
TA97	0	147 ± 11.0	179 ± 6.0	155 ± 6.9	185 ± 11.6	150 ± 4.9	155 ± 16.2
	100	152 ± 15.9	156 ± 16.6	134 ± 5.2	181 ± 15.8	166 ± 3.1	148 ± 20.9
	333	144 ± 4.6	187 ± 8.1	155 ± 15.5	172 ± 26.6	168 ± 4.4	155 ± 4.2
	1,000	150 ± 13.7	186 ± 7.3	135 ± 12.9	200 ± 10.9	175 ± 7.3	147 ± 5.6
	3,333	127 ± 17.2	170 ± 13.2	132 ± 2.0	202 ± 18.6	165 ± 11.4	176 ± 9.6
	6,666	99 ± 19.0[c]	90 ± 40.7[c]		175 ± 14.0		
	10,000			84 ± 2.1[c]		173 ± 8.1	171 ± 8.7
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		768 ± 102.2	773 ± 52.6	395 ± 17.5	344 ± 6.7	336 ± 3.2	340 ± 18.9
TA98	0	17 ± 0.9	28 ± 5.1	22 ± 2.3	54 ± 0.3	22 ± 1.5	45 ± 1.7
	100	17 ± 2.9	29 ± 2.0	30 ± 2.3	39 ± 5.5	21 ± 3.2	36 ± 4.0
	333	15 ± 1.2	26 ± 3.5	28 ± 1.5	41 ± 4.8	16 ± 1.5	30 ± 5.5
	1,000	15 ± 1.0	29 ± 2.1	29 ± 2.9	28 ± 7.7	19 ± 3.2	28 ± 6.9
	3,333	16 ± 0.3	26 ± 0.7	21 ± 3.8[c]	36 ± 3.3[c]	20 ± 2.7	23 ± 3.2
	6,666	13 ± 1.5			49 ± 2.4[c]		
	10,000		Toxic	17 ± 2.3[c]		17 ± 0.9	39 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		508 ± 7.0	534 ± 20.5	363 ± 10.8	449 ± 22.3	255 ± 23.2	157 ± 9.3

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

Strain	Dose (µg/plate)	Revertants/Plate −S9 Trial 1	−S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
Study performed at Microbiological Associates, Inc.							
TA100	0	127 ± 12.9	95 ± 5.8	85 ± 2.2	117 ± 5.3	101 ± 5.8	142 ± 5.2
	33	125 ± 4.2	90 ± 7.5	80 ± 3.2	117 ± 8.8	92 ± 1.5	132 ± 2.3
	100	115 ± 6.8	101 ± 4.7	76 ± 2.8	118 ± 3.9	89 ± 1.5	138 ± 3.4
	333	132 ± 8.1	91 ± 3.5	82 ± 2.6	108 ± 7.1	98 ± 7.0	134 ± 7.0
	1,000	120 ± 4.3	89 ± 6.3	78 ± 3.8	108 ± 5.3	88 ± 4.5	130 ± 0.9
	2,000	80 ± 3.8			101 ± 1.2		100 ± 3.2
	3,333		80 ± 12.3[c]	63 ± 2.9[c]		74 ± 4.1[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		336 ± 15.1	416 ± 14.8	567 ± 24.8	457 ± 18.6	624 ± 2.7	686 ± 22.0
TA1535	0	19 ± 4.0	19 ± 1.7	8 ± 1.7	10 ± 1.7	8 ± 1.7	11 ± 1.8
	33	18 ± 3.8	23 ± 1.5	9 ± 1.5	10 ± 0.7	9 ± 3.3	12 ± 1.0
	100	17 ± 3.4	25 ± 4.5	9 ± 0.7	10 ± 1.7	12 ± 3.1	9 ± 0.9
	333	19 ± 2.3	22 ± 3.4	7 ± 1.3	9 ± 1.7	11 ± 1.0	9 ± 1.2
	1,000	15 ± 0.9	20 ± 0.7	8 ± 2.2	13 ± 0.6	12 ± 1.5	12 ± 2.2
	2,500	14 ± 1.2			7 ± 0.3		11 ± 1.9
	3,333		14 ± 1.0[c]	5 ± 2.3[c]		7 ± 0.9[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		210 ± 15.3	272 ± 3.0	57 ± 3.2	92 ± 2.0	127 ± 7.0	135 ± 17.4
TA1537	0	6 ± 0.7			6 ± 0.0		4 ± 0.3
	33	6 ± 0.6			7 ± 1.0		7 ± 0.6
	100	3 ± 1.2			7 ± 0.6		8 ± 1.2
	333	4 ± 1.5			3 ± 0.7		6 ± 1.7
	1,000	5 ± 2.0			4 ± 1.2		6 ± 2.7
	2,500	6 ± 1.2[c]			6 ± 1.5		6 ± 3.6
Trial summary		Negative			Negative		Negative
Positive control		36 ± 1.5			217 ± 3.4		78 ± 5.5
TA97	0	70 ± 8.4	93 ± 5.6	98 ± 17.0	114 ± 5.2	119 ± 10.8	137 ± 3.0
	33	65 ± 10.2	89 ± 5.5	100 ± 4.0	117 ± 3.7	115 ± 12.4	149 ± 8.4
	100	74 ± 7.7	79 ± 10.0	105 ± 9.0	124 ± 6.9	117 ± 2.6	152 ± 6.9
	333	79 ± 1.2	87 ± 5.0	112 ± 1.7	120 ± 4.7	118 ± 14.9	133 ± 10.2
	1,000	81 ± 6.4	81 ± 1.5	111 ± 4.3	125 ± 7.5	117 ± 6.7	129 ± 8.1
	2,500	56 ± 2.6[c]			98 ± 4.0[c]		97 ± 4.5[c]
	3,333		74 ± 7.6[c]	78 ± 4.9[c]		80 ± 7.4[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		208 ± 26.6	187 ± 9.5	783 ± 36.5	988 ± 40.3	1,409 ± 47.4	577 ± 6.7

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

Strain	Dose (µg/plate)	Revertants/Plate					
		−S9		+hamster S9		+rat S9	
		Trial 1	Trial 2	10%	30%	10%	30%
TA98	0	15 ± 1.5	15 ± 3.1	33 ± 2.2	21 ± 1.5	26 ± 0.9	21 ± 3.0
	33	11 ± 2.3	15 ± 1.5	27 ± 3.1	23 ± 5.8	19 ± 1.7	26 ± 1.0
	100	17 ± 4.4	11 ± 3.2	28 ± 2.5	21 ± 2.6	21 ± 4.4	21 ± 1.7
	333	14 ± 3.5	14 ± 1.7	26 ± 1.5	24 ± 0.6	22 ± 2.4	23 ± 2.6
	1,000	9 ± 1.0	11 ± 1.0	27 ± 2.9	27 ± 1.7	19 ± 4.4	17 ± 2.2
	2,000	11 ± 0.3			23 ± 2.7		17 ± 0.6
	3,333		13 ± 2.3[c]	19 ± 0.9[c]		18 ± 4.2[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		116 ± 0.6	209 ± 5.0	233 ± 7.1	78 ± 11.6	259 ± 26.9	151 ± 4.2

[a] The detailed protocol and these data are presented by Zeiger *et al.* (1988). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537 and TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Mutagenicity of *trans*-1,2-Dichloroethylene in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] −S9 Trial 1	−S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0.0	106 ± 4.7	88 ± 4.6	100 ± 4.9	82 ± 8.9	108 ± 7.9	99 ± 4.3
	33.3	109 ± 2.6		99 ± 18.2		97 ± 3.5	
	100.0	113 ± 8.1	99 ± 6.3	104 ± 4.9	92 ± 6.3	97 ± 4.9	109 ± 4.6
	333.3	108 ± 9.0	80 ± 6.7	109 ± 5.0	104 ± 7.8	98 ± 5.2	94 ± 2.2
	1,000.0	102 ± 12.8	82 ± 8.4	89 ± 5.1	88 ± 5.8	94 ± 8.4	95 ± 11.1
	3,333.3	114 ± 5.2	72 ± 2.7[c]	94 ± 2.2	76 ± 8.1	100 ± 5.7	79 ± 6.5[c]
	10,000.0		Toxic		78 ± 10.0[c]		85 ± 4.2[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		457 ± 14.6	253 ± 7.5	1,423 ± 23.4	1,587 ± 36.5	1,214 ± 55.4	514 ± 8.0
TA1535	0.0	19 ± 2.6	26 ± 2.9	11 ± 1.5	11 ± 1.5	9 ± 0.3	15 ± 1.2
	33.3	20 ± 0.3		13 ± 0.7		12 ± 1.8	
	100.0	18 ± 1.5	20 ± 2.5	12 ± 1.2	13 ± 2.8	14 ± 0.6	15 ± 2.1
	333.3	18 ± 1.7	19 ± 4.7	10 ± 1.5	13 ± 0.6	9 ± 2.3	12 ± 3.2
	1,000.0	18 ± 2.5	24 ± 4.6	8 ± 2.6	12 ± 2.8	10 ± 1.2	9 ± 2.5
	3,333.3	24 ± 4.5	19 ± 3.8	8 ± 0.7	12 ± 1.2	10 ± 1.8	12 ± 0.6
	10,000.0		18 ± 1.8		7 ± 1.0		11 ± 1.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		385 ± 21.5	542 ± 14.1	513 ± 5.8	639 ± 37.9	479 ± 17.8	303 ± 7.4
TA1537	0.0	7 ± 1.2	9 ± 0.3	29 ± 8.0	21 ± 4.4	24 ± 3.4	15 ± 2.9
	33.3	7 ± 1.5		26 ± 4.6		18 ± 0.9	
	100.0	13 ± 3.4	8 ± 0.0	25 ± 3.3	24 ± 4.0	18 ± 3.8	20 ± 0.6
	333.3	11 ± 2.3	12 ± 0.9	22 ± 3.7	23 ± 1.7	16 ± 0.7	13 ± 2.9
	1,000.0	8 ± 1.3	13 ± 1.2	28 ± 6.9	22 ± 2.2	16 ± 4.3	17 ± 2.0
	3,333.3	6 ± 2.0	9 ± 1.5	21 ± 4.4	18 ± 0.7	13 ± 1.2	13 ± 2.1
	10,000.0		7 ± 0.0		18 ± 1.0		18 ± 4.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		251 ± 29.2	222 ± 6.1	363 ± 32.1	394 ± 36.0	409 ± 15.6	237 ± 14.1
TA98	0.0	26 ± 3.6	22 ± 4.7	55 ± 7.1	35 ± 2.5	61 ± 2.9	39 ± 2.3
	33.3	34 ± 3.2		48 ± 1.5		52 ± 2.1	
	100.0	34 ± 3.0	16 ± 1.2	56 ± 6.8	32 ± 4.3	56 ± 3.9	39 ± 0.7
	333.3	34 ± 2.7	21 ± 2.4	54 ± 1.2	29 ± 2.0	61 ± 6.4	41 ± 0.6
	1,000.0	33 ± 3.8	22 ± 2.0	49 ± 3.2	32 ± 5.0	54 ± 5.4	38 ± 3.0
	3,333.3	23 ± 4.4	23 ± 6.5	45 ± 3.6	30 ± 1.0	41 ± 2.3	39 ± 1.5
	10,000.0		21 ± 3.5		35 ± 1.2		37 ± 4.1
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		681 ± 18.0	682 ± 36.1	1,513 ± 57.8	1,605 ± 124.9	440 ± 27.3	489 ± 4.7

[a] Study was performed at SRI International. The detailed protocol and these data are presented by Mortelmans *et al.* (1986). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E3
Mutagenicity of *cis,trans*-1,2-Dichloroethylene 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
TA100	0.0	85 ± 6.6	107 ± 4.2	84 ± 6.2	78 ± 5.8	96 ± 10.0	94 ± 3.7
	33.3	80 ± 4.8	78 ± 1.2	100 ± 7.6	85 ± 4.5	77 ± 5.5	82 ± 3.5
	100.0	64 ± 5.2	75 ± 3.8	94 ± 4.4	92 ± 3.5	78 ± 6.9	88 ± 0.9
	333.3	83 ± 8.5	85 ± 7.8	85 ± 5.6	83 ± 4.5	78 ± 5.0	82 ± 6.8
	1,000.0	72 ± 10.0	63 ± 6.3	82 ± 5.8	66 ± 3.8	79 ± 6.4	72 ± 5.5
	3,333.3	73 ± 7.8[c]	44 ± 2.8[c]	88 ± 7.1	59 ± 4.3[c]	66 ± 2.8[c]	51 ± 1.5[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		413 ± 8.8	350 ± 23.3	1,383 ± 66.9	1,991 ± 73.8	528 ± 9.3	807 ± 30.1
TA1535	0.0	10 ± 1.3	13 ± 3.0	6 ± 2.5	7 ± 2.7	9 ± 1.5	7 ± 1.3
	33.3	18 ± 1.2	5 ± 0.0	8 ± 0.6	5 ± 0.9	7 ± 1.2	5 ± 2.0
	100.0	6 ± 0.9	7 ± 0.7	9 ± 2.7	6 ± 1.3	10 ± 3.5	6 ± 1.8
	333.3	11 ± 3.8	6 ± 0.6	7 ± 1.2	4 ± 0.3	9 ± 1.7	4 ± 1.2
	1,000.0	9 ± 3.1	7 ± 1.2	5 ± 0.9	5 ± 0.3	6 ± 3.0	4 ± 0.6
	3,333.3	6 ± 4.2[c]	4 ± 1.8	5 ± 1.3	4 ± 1.0	6 ± 0.3	3 ± 0.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		350 ± 10.4	353 ± 18.9	470 ± 34.0	413 ± 24.4	313 ± 14.2	288 ± 21.2
TA1537	0.0	7 ± 1.5	3 ± 0.9	7 ± 3.1	5 ± 1.2	9 ± 2.1	6 ± 1.5
	33.3	6 ± 0.9	4 ± 0.9	8 ± 0.9	3 ± 0.0	5 ± 0.3	5 ± 1.2
	100.0	7 ± 1.0	3 ± 0.3	9 ± 3.3	6 ± 1.0	6 ± 0.0	3 ± 0.6
	333.3	5 ± 0.9	3 ± 1.2	6 ± 0.9	4 ± 1.3	5 ± 0.0	4 ± 0.3
	1,000.0	4 ± 0.6	2 ± 0.6	5 ± 0.9	4 ± 0.3	7 ± 1.7	4 ± 0.3
	3,333.3	3 ± 0.9[c]	2 ± 0.9[c]	4 ± 0.3	3 ± 1.7[c]	4 ± 0.9[c]	3 ± 0.3[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		124 ± 40.2	164 ± 42.6	445 ± 13.0	397 ± 37.6	235 ± 5.8	189 ± 15.9
TA98	0.0	28 ± 1.7	20 ± 2.7	24 ± 1.7	31 ± 0.9	35 ± 5.9	30 ± 1.7
	33.3	15 ± 0.9	16 ± 1.7	40 ± 9.7	26 ± 2.4	37 ± 4.1	25 ± 1.9
	100.0	24 ± 3.8	20 ± 0.6	30 ± 3.4	34 ± 3.6	48 ± 8.0	23 ± 3.4
	333.3	21 ± 3.2	20 ± 0.9	31 ± 4.3	36 ± 1.7	34 ± 2.3	26 ± 1.2
	1,000.0	16 ± 1.9	11 ± 1.9	26 ± 3.8	30 ± 1.2	23 ± 3.3	13 ± 2.6
	3,333.3	7 ± 1.5[c]	3 ± 1.2[c]	25 ± 2.9	19 ± 1.9[c]	14 ± 2.6[c]	8 ± 2.2[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		797 ± 18.8	784 ± 38.8	1,177 ± 53.5	1,664 ± 90.1	326 ± 16.1	584 ± 14.2

[a] Study was performed at SRI International. The detailed protocol and these data are presented by Mortelmans *et al.* (1986). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E4
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *cis*-1,2-Dichloroethylene[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								
Trial 1								
Summary: Positive								
Dimethylsulfoxide[c]		50	1,050	396	0.37	7.9	26.0	
cis-1,2-Dichloroethylene								
	50	50	1,052	452	0.42	9.0	26.0	13.92
	160	50	1,050	438	0.41	8.8	26.0	10.61
	500	50	1,049	539	0.51	10.8	26.0	36.24*
	1,600	50	1,046	521	0.49	10.4	26.0	32.07*
	5,000	9[d]	188	91	0.48	10.1	26.0	28.34*
					P<0.001[e]			
Mitomycin-C[f]	0.0005	50	1,051	516	0.49	10.3	26.0	30.18
	0.005	10	210	311	1.48	31.1	26.0	292.68
Trial 2								
Summary: Weakly positive								
Dimethylsulfoxide		50	1,050	463	0.44	9.3	26.0	
cis-1,2-Dichloroethylene								
	160	50	1,044	471	0.45	9.4	26.0	2.31
	500	50	1,048	525	0.50	10.5	26.0	13.61
	1,600	50	1,050	546	0.52	10.9	26.0	17.93
	5,000	50	1,046	591	0.56	11.8	26.0	28.13*
					P<0.001			
Mitomycin-C	0.0007	50	1,046	589	0.56	11.8	26.0	27.70
	0.005	10	210	296	1.40	29.6	26.0	219.66

TABLE E4
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *cis*-1,2-Dichloroethylene

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 (%)
+S9								
Summary: Equivocal								
Dimethylsulfoxide		50	1,050	441	0.42	8.8	26.5	
cis-1,2-Dichloroethylene								
	160	50	1,049	450	0.42	9.0	26.5	2.14
	500	50	1,050	462	0.44	9.2	26.5	4.76
	1,600	50	1,049	495	0.47	9.9	26.5	12.35
	5,000	50	1,047	523	0.49	10.5	26.5	18.93
					P=0.001			
Cyclophosphamide[f]	0.1	50	1,049	550	0.52	11.0	26.5	24.83
	0.6	10	210	207	0.98	20.7	26.5	134.69

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

TABLE E5
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *trans*-1,2-Dichloroethylene[a]

Compound	Concentration (µg/mL)	Total Cells Scored	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
–S9								
Summary: Negative								
Dimethylsulfoxide[c]		50	1,049	428	0.40	8.6	26.0	
trans-1,2-Dichloroethylene								
	160	50	1,042	460	0.44	9.2	26.0	8.20
	500	50	1,039	459	0.44	9.2	26.0	8.28
	1,600	50	1,039	474	0.45	9.5	26.0	11.81
	5,000	50	1,044	416	0.39	8.3	26.0	–2.34
					P=0.526[d]			
Mitomycin-C[e]	0.0005	50	1,049	561	0.53	11.2	26.0	31.08
	0.005	10	210	297	1.41	29.7	26.0	246.64
+S9								
Summary: Equivocal								
Dimethylsulfoxide		50	1,050	388	0.36	7.8	26.0	
trans-1,2-Dichloroethylene								
	160	50	1,049	414	0.39	8.3	26.0	6.80
	500	50	1,050	451	0.42	9.0	26.0	16.24
	1,600	50	1,050	457	0.43	9.1	26.0	17.78
	5,000	50	1,048	453	0.43	9.1	26.0	16.98
					P=0.004			
Cyclophosphamide[e]	0.1	50	1,050	537	0.51	10.7	26.0	38.40
	0.6	10	210	226	1.07	22.6	26.0	191.24

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

TABLE E6
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *cis,trans*-1,2-Dichloroethylene[a]

Compound	Concentration (µg/mL)	Total Cells Scored	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
−S9								
Summary: Positive								
Dimethylsulfoxide[c]		50	1,041	411	0.39	8.2	25.8	
cis,trans-1,2-Dichloroethylene								
	126	50	1,027	523	0.50	10.5	25.8	28.99*
	421	50	1,041	621	0.59	12.4	25.8	51.10*
	1,263	50	1,041	871	0.83	17.4	25.8	111.92*
					P<0.001[d]			
Mitomycin-C[e]	0.005	50	1,036	1,347	1.30	26.9	25.8	229.32
+S9								
Summary: Positive								
Dimethylsulfoxide		50	1,027	427	0.41	8.5	25.5	
cis,trans-1,2-Dichloroethylene								
	1,263	50	1,037	618	0.59	12.4	25.5	43.33*
	4,210[f]	50	1,021	1,912	1.87	38.2	25.5	350.41*
	12,630[f]	50	1,025	2,545	2.48	50.9	25.5	497.19*
					P<0.001			
Cyclophosphamide[e]	1.5	50	1,032	2,234	2.16	44.7	25.5	420.65

* Positive response (≥20% increase over solvent control)
[a] Study was performed at Litton Bionetics, Inc. The detailed protocol is presented by Galloway *et al.* (1987). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c] Solvent control
[d] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose
[e] Positive control
[f] Etching of the plastic tissue culture flasks occurred at this dose.

TABLE E7
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *cis*-1,2-Dichloroethylene[a]

Compound	Concentration (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
−S9					
Harvest time: 12.0 hours					
Summary: Negative					
Dimethylsulfoxide[b]		200	2	0.01	1.0
cis-1,2-Dichloroethylene	500	200	3	0.02	1.5
	1,000	200	3	0.02	1.5
	1,600	200	3	0.02	1.5
					P=0.340[c]
Mitomycin-C[d]	0.0625	200	34	0.17	13.0
	0.25	50	20	0.40	36.0
+S9					
Harvest time: 13.0 hours					
Summary: Negative					
Dimethylsulfoxide		200	2	0.01	1.0
cis-1,2-Dichloroethylene	1,600	200	6	0.03	2.5
	3,000	200	11	0.06	3.0
	5,000	200	2	0.01	1.0
					P=0.433
Cyclophosphamide[d]	2.5	200	34	0.17	14.5
	7.5	50	21	0.42	36.0

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Galloway *et al.* (1987).
[b] Solvent control
[c] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[d] Positive control

TABLE E8
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *trans*-1,2-Dichloroethylene[a]

Compound	Concentration (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
−S9					
Harvest time: 12.0 hours					
Summary: Negative					
Dimethylsulfoxide[b]		200	1	0.01	0.5
trans-1,2-Dichloroethylene	1,600	200	0	0.00	0.0
	3,000	200	1	0.01	0.5
	5,000	200	1	0.01	0.5
					P=0.394[c]
Mitomycin-C[d]	0.0625	200	36	0.18	17.0
	0.25	50	15	0.30	18.0
+S9					
Harvest time: 13.0 hours					
Summary: Negative					
Dimethylsulfoxide		200	0	0.00	0.0
trans-1,2-Dichloroethylene	1,600	200	1	0.01	0.5
	3,000	200	3	0.02	1.5
	5,000	200	2	0.01	1.0
					P=0.066
Cyclophosphamide[d]	2.5	200	34	0.17	16.0
	7.5	50	21	0.42	22.0

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Galloway *et al.* (1987).
[b] Solvent control
[c] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[d] Positive control

TABLE E9
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *cis,trans*-1,2-Dichloroethylene[a]

Compound	Concentration (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
−S9					
Harvest time: 22.5 hours[b]					
Summary: Negative					
Dimethylsulfoxide[c]		100	3	0.03	3.0
cis,trans-1,2-Dichloroethylene	594	100	5	0.05	5.0
	1,184	50[d]	2	0.04	2.0
	2,374[e]	100	4	0.04	3.0
	4,798[e]	0[f]			
	9,496[e]	100	10	0.10	9.0
					P=0.040[g]
Mitomycin-C[h]	500	100	25	0.25	21.0
+S9					
Trial 1					
Harvest time: 10.5 hours					
Summary: Negative					
Dimethylsulfoxide		100	5	0.05	5.0
cis,trans-1,2-Dichloroethylene	772	100	24	0.24	13.0
	1,543[e]	100	6	0.06	6.0
	3,086[e]	0[f]			
	6,173[e]	100	5	0.05	3.0
	9,496[e]	100	5	0.05	5.0
	12,630[e]	100	6	0.06	5.0
					P=0.941
Cyclophosphamide[h]	50	100	53	0.53	29.0

TABLE E9
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *cis,trans*-1,2-Dichloroethylene

Compound	Concentration (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
+S9 (continued)					
Trial 2					
Harvest time: 10.5 hours					
Summary: Negative					
Dimethylsulfoxide		100	6	0.06	4.0
cis,trans-1,2-Dichloroethylene	455	100	15	0.15	10.0
	607	100	7	0.07	6.0
	758	100	12	0.12	3.0
	910[e]	100	6	0.06	6.0
	1,210[e]	100	12	0.12	8.0
	1,516[e]	100	10	0.10	8.0
					P=0.277
Cyclophosphamide	50	100	45	0.45	28.0

[a] Study was performed at Litton Bionetics, Inc. The detailed protocol is 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] Incomplete count due to the poor quality of this culture
[e] Etching of the plastic tissue culture flasks occurred at this dose.
[f] Severe toxicity
[g] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[h] Positive control

TABLE E10
Induction of Sister Chromatid Exchanges in Bone Marrow Cells of Male Mice by *cis*-1,2-Dichloroethylene[a]

Compound	Dose (mg/kg)	SCEs/Cell
Sample time: 23 hours		
Corn oil[b]		3.36 ± 0.43
cis-1,2-Dichloroethylene	500	3.43 ± 0.37
	1,000	4.65 ± 0.45
	2,000	3.83 ± 0.58
Dimethylbenzanthracene[c]	100	13.29 ± 0.95

[a] Study was performed at Oak Ridge Associated Universities. The detailed protocol is presented by Tice *et al.* (1987); 25 second-division metaphase cells were scored from each of four mice per group. Data for SCEs/cell are given as mean ± standard error. SCE=sister chromatid exchange
[b] Vehicle control
[c] Positive control

TABLE E11
Induction of Sister Chromatid Exchanges in Bone Marrow Cells of Male Mice by *trans*-1,2-Dichloroethylene[a]

Compound	Dose (mg/kg)	SCEs/Cell
Sample time: 23 hours		
Corn oil[b]		3.36 ± 0.43
trans-1,2-Dichloroethylene	500	3.55 ± 0.29
	1,000	3.38 ± 0.20
	2,000	3.00 ± 0.11
Dimethylbenzanthracene[c]	100	13.29 ± 0.95

[a] Study was performed at Oak Ridge Associated Universities. The detailed protocol is presented by Tice *et al.* (1987); 25 second-division metaphase cells were scored from each of four mice per group. Data for SCEs/cell are given as mean ± standard error. SCE=sister chromatid exchange
[b] Vehicle control
[c] Positive control

TABLE E12
Induction of Chromosomal Aberrations in Bone Marrow Cells of Male Mice by *cis*-1,2-Dichloroethylene[a]

	Dose (mg/kg)	Number of Mice with Cells Scored	Cells with Aberrations[b]	P Value
Sample time: 17 hours				
Corn oil[c]		8	2.25 ± 0.45	
cis-1,2-Dichloroethylene	500	8	1.50 ± 0.50	0.7829
	1,000	8	1.50 ± 0.73	0.7829
	2,000	7	2.29 ± 0.81	0.4869
			P=0.430[d]	
Dimethylbenzanthracene[e]	100	8	21.00 ± 2.85	0.0000

[a] Study was performed at Oak Ridge Associated Universities. The detailed protocol is presented by Tice *et al.* (1987); 50 first-division metaphase cells were scored from each of seven or eight mice per group.
[b] Mean ± standard error
[c] Vehicle control
[d] Significance tested by a one-tailed trend test (Margolin *et al.*, 1986)
[e] Positive control

TABLE E13
Induction of Chromosomal Aberrations in Bone Marrow Cells of Male Mice by *trans*-1,2-Dichloroethylene[a]

	Dose (mg/kg)	Number of Mice with Cells Scored	Cells with Aberrations[b]	P Value
Sample time: 17 hours				
Corn oil[c]		8	2.25 ± 0.45	
trans-1,2-Dichloroethylene	500	8	1.75 ± 1.03	0.6367
	1,000	8	3.75 ± 1.91	0.1947
	2,000	8	1.75 ± 0.80	0.6367
			P=0.546[d]	
Dimethylbenzanthracene[e]	100	8	21.00 ± 2.85	0.0000

[a] Study was performed at Oak Ridge Associated Universities. The detailed protocol is presented by Tice *et al.* (1987); 50 first-division metaphase cells were scored from each of eight mice per group.
[b] Mean ± standard error
[c] Vehicle control
[d] Significance tested by a one-tailed trend test (Margolin *et al.*, 1986)
[e] Positive control

TABLE E14
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of *trans*-1,2-Dichloroethylene in Feed for 14 Weeks[a]

Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs [b]	P Value[c]	PCEs (%)
Male				
Untreated Control	10	1.15 ± 0.20		1.6
Vehicle Control	10	1.05 ± 0.16		1.4
3,125	10	1.05 ± 0.23	0.6186	1.4
6,250	10	0.95 ± 0.16	0.7316	1.5
12,500	10	1.05 ± 0.16	0.6186	1.7
25,000	10	0.70 ± 0.17	0.9306	1.7
50,000	10	0.75 ± 0.21	0.9029	1.9
		P=0.936[d]		
Female				
Untreated Control	10	0.85 ± 0.18		1.7
Vehicle Control	10	0.90 ± 0.21		1.7
3,125	10	0.65 ± 0.11	0.7675	1.9
6,250	10	0.95 ± 0.22	0.3694	1.7
12,500	10	0.85 ± 0.13	0.5000	1.8
25,000	10	0.75 ± 0.19	0.6382	1.8
50,000	10	0.70 ± 0.15	0.7051	1.9
		P=0.699		

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

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF *TRANS*-1,2-DICHLOROETHYLENE

trans-1,2-Dichloroethylene was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI), in one lot (MP-02224LP) for use in the 14-week feed studies. The chemical was microencapsulated by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the microcapsules were assigned a separate lot number (343-1B-A). Identity, purity, and stability analyses of the neat and microencapsulated chemical were conducted by the analytical chemistry laboratory and the study laboratory. Reports on analyses performed in support of the *trans*-1,2-dichloroethylene 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 *trans*-1,2-dichloroethylene by the analytical chemistry laboratory with infrared, ultraviolet/visible, and nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory with infrared spectroscopy. The infrared and NMR spectra were consistent with literature references (*Sadtler Standard Spectra; High Resolution NMR Spectra Catalog*, 1963). All spectra were consistent with the structure of *trans*-1,2-dichloroethylene. The infrared and NMR spectra are presented in Figures F1 and F2.

The purity of *trans*-1,2-dichloroethylene was determined by the analytical chemistry laboratory using elemental analyses, Karl Fischer water analysis, free acid titration, and gas chromatography (GC). To measure the concentration of free acid, *trans*-1,2-dichloroethylene was dissolved in methanol and titrated with 0.01 N sodium hydroxide to the phenolphthalein endpoint. GC analyses were performed with systems A and B as described in Table F1. Additional GC analyses with mass spectrometry (GC/MS) were performed to determine whether selected chlorinated impurities were present and to quantify any impurities that were detected. The study laboratory analyzed purity using GC by system C.

Elemental analyses for carbon, hydrogen, and chlorine were in agreement with the theoretical values for *trans*-1,2-dichloroethylene. Karl Fischer water analysis indicated 0.010% ± 0.001% water. Free acid titration indicated 0.0007 ± 0.0002 mEq acid per gram, equivalent to 26 ± 7 ppm as hydrochloric acid. GC by system A indicated one major peak and five impurities with a combined area of 1.25% relative to the major peak area. GC by system B indicated one major peak and three impurities with a combined relative area of 0.69%. The overall purity of lot MP-02224LP was determined to be approximately 99%. GC/MS analyses by systems D and E or similar systems identified nine chlorinated compounds: *cis*-1,2-dichloroethylene (1,600 ppm), methylene chloride (300 ppm), 1,1- and 1,2-dichloroethane (300 and approximately 2 ppm, respectively), chloroform (82 ppm), trichloroethylene (12 ppm), and tetrachloroethylene, chlorobenzene, and vinyl chloride (less than 1 ppm each). Additionally, carbon tetrachloride and 1,1,2,2-tetrachloroethane were tentatively identified at concentrations less than 1 ppm. The study laboratory confirmed that the purity was 99% or greater using GC by system C.

Stability information supplied by the manufacturer indicated that neat *trans*-1,2-dichloroethylene is stable when stored frozen and protected from air, moisture, and light; the chemical was stored under these conditions throughout the 14-week studies. Stability was monitored during preliminary pilot studies conducted by TSI Mason Laboratories (Worcester, MA) with infrared spectroscopy and GC by systems similar to system C; no degradation of the bulk chemical was detected.

Microcapsule Formulation and Analyses

Microcapsules loaded with neat *trans*-1,2-dichloroethylene and placebos (empty microcapsules) were prepared by the analytical chemistry laboratory with a proprietary process using food-grade, modified corn starch (CAPSUL®) 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, 120, and PAN). The chemical loads (amount of *trans*-1,2-dichloroethylene in the starch/sugar matrix) of freshly prepared microcapsules and of microcapsules stored under a variety of conditions were determined with GC by systems similar to system C. Samples for GC analysis were prepared by dissolving the microcapsules in 50 mL of a 60:40 methanol:water solution by shaking for 15 minutes; 50 mL of an internal standard solution (4 mg/mL 1,1,1-trichloroethane in methanol) were added, and the mixtures were shaken and filtered. Major peak comparisons of the neat and microencapsulated chemicals and 9-month stability studies were also performed with GC by systems similar to system C.

Microscopic examination revealed no unusual characteristics. The particles were within size specifications. The mean chemical load was determined to be 45.0% ± 0.3%. Microcapsules exposed to animal room conditions (open to air and light) for 28 days retained 93.8% of their chemical load by weight; additional samples similarly exposed after seven freeze-thaw cycles retained 93% of their chemical load after 28 days. Microcapsules stored in sealed bottles at 5° C for 28 days retained 95% of their chemical load by weight. Major peak comparisons indicated that no impurities were introduced by microencapsulation; one impurity occurring in the neat chemical with a relative area of 0.24% was not observed in the encapsulated chemical. Results of the 9-month shelf-life study indicated that microcapsules retained 91% of their chemical load when stored at 5° C and 89% when stored at room temperature; microcapsules stored at 5° C for 6 months and then at room temperature, open to air and light, for 28 days were stable.

The study laboratory confirmed the identity of the microcapsules with infrared spectroscopy and analyzed the chemical load of the microcapsules using GC by a system similar to system F. GC analyses performed at the beginning of the preliminary pilot studies indicated a chemical load of approximately 43%. Prior to the 14-week studies, GC analyses of *trans*-1,2-dichloroethylene samples from one bottle of microcapsules indicated a concentration of 37.6% ± 2.4%, which was lower than the expected concentration of 45.3%; microcapsules from six other bottles were mixed in a twin-shell blender with the intensifier bar on for 5 minutes and off for 10 minutes. Samples were extracted for 15 minutes with 50 mL methanol:water (60:40); 50 mL methanol were added, and the samples were extracted for an additional minute. The extract was combined with the internal standard, 200 ppm 1,1,1-trichloroethane in 5% methanol:hexane (1:1), and diluted to 40 mL with hexane. The mixture was shaken for 1 minute, and an aliquot of the upper hexane phase was analyzed with GC by system G. Homogeneity was confirmed, and the chemical load of 47.0% was considered to be acceptable for use in the 14-week studies. The chemical load was monitored during the 14-week studies with GC by system G; no loss of *trans*-1,2-dichloroethylene was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared at least every 2 weeks by mixing microencapsulated *trans*-1,2-dichloroethylene with feed (Table F2). Placebo microcapsules were added to maintain a starch matrix concentration of 6% in the diet. A premix was prepared by hand and then blended with additional feed in a twin-shell blender for 15 minutes, with the intensifier bar on for the first 5 minutes. The dose formulations were then kneaded and mixed manually, then mixed for an additional 15 minutes in the blender, with the

intensifier bar on for the first 5 minutes. Formulations were stored in doubled plastic bags, protected from light, at 5° C for up to 4 weeks.

Homogeneity and stability studies of 0.5% *trans*-1,2-dichloroethylene formulations were conducted by the analytical chemistry laboratory using GC by system G or similar systems. Homogeneity was confirmed. The *trans*-1,2-dichloroethylene formulation prepared with lot 343-1A had losses of 22.0% for samples stored for 7 days in the dark and 15.5% for samples stored for 1 day open to air and light; dose formulations prepared with lots 343-10TA, -11TA, and -12TA (not used in the current studies) and 343-1B-A were stable for 4 days when stored at room temperature, open to air and light. A loss of 5.2% for the *trans* isomer was observed after 3 weeks for dose formulations stored in the dark at room temperature. Additional analyses performed with GC by system F with a 0.5% dose formulation prepared with lot 343-1B-A confirmed stability for 7 days for samples stored in a rat cage, open to air and light, at up to 50% humidity.

Prior to the 14-week studies, the study laboratory performed homogeneity studies of the 3,125 and 50,000 ppm dose formulations and stability studies of the 3,125 ppm dose formulation with GC by a system similar to system G. Homogeneity of all formulations was confirmed. Stability was confirmed for 28 days for dose formulations stored in plastic bags at up to 5° C. Dose formulations were stored refrigerated in doubled plastic bags during the studies.

Periodic analyses of the dose formulations were conducted by the study laboratory using GC by systems similar to system C. The first four sets of dose formulations, as well as those at the midpoint and end of the studies, were analyzed; animal room samples of the dose formulations taken from feeders on the last dosing day prior to the expiration date of the batch were also analyzed at the beginning, midpoint, and end of the studies (Table F3). The results for the initial analyses of the first two sets of dose formulations and the midpoint dose formulations were high; additional feed was added, and these dose formulations were reblended and reanalyzed. All were found to be within 10% of the target concentrations. The concentration of one dose formulation from the fourth set was slightly high; this dose formulation was considered acceptable for use. Two of the final set of dose formulations also were not within 10% of the target concentrations; these were remixed, and the remixes were found to be within specifications. The concentrations of all animal room samples except one sample for rats were more than 10% lower than the target concentrations; this was considered to be the result of degradation of the microcapsule matrix by condensation of atmospheric moisture during frozen storage and possibly by contamination of the feed with urine.

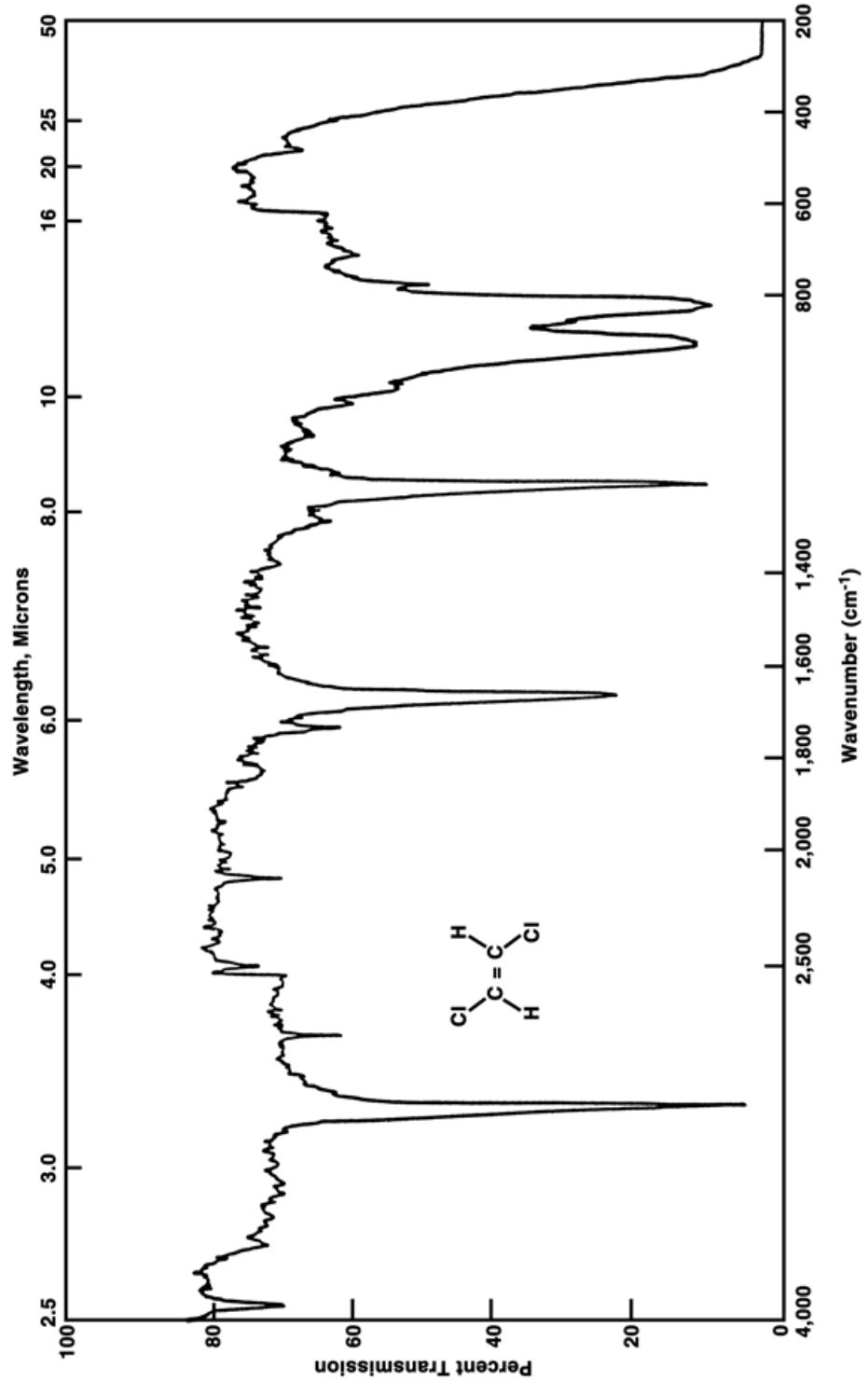

FIGURE F1
Infrared Absorption Spectrum of *trans*-1,2-Dichloroethylene

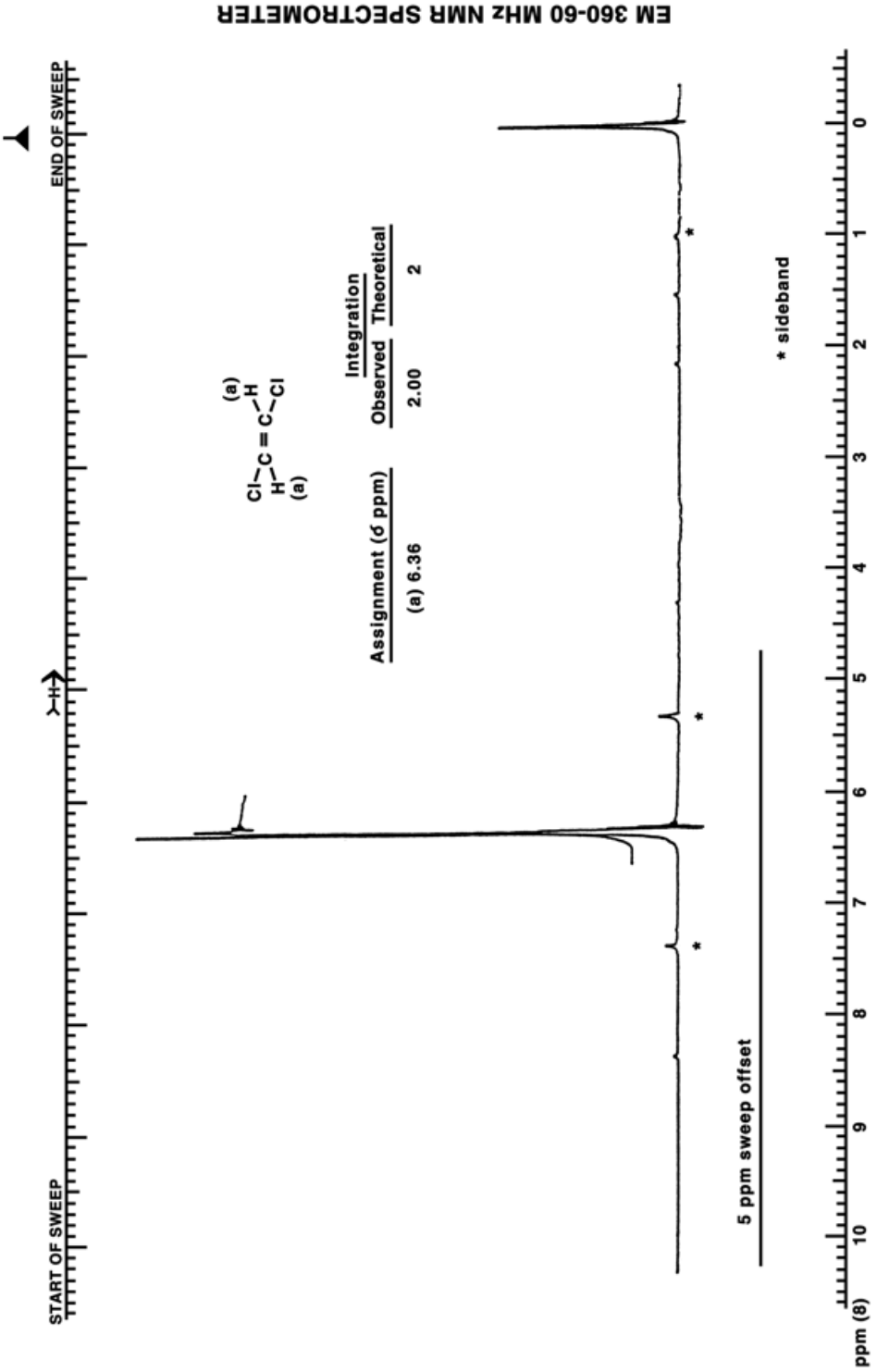

FIGURE F2
Nuclear Magnetic Resonance Spectrum of *trans*-1,2-Dichloroethylene

TABLE F1
Gas Chromatography Systems Used in the 14-Week Feed Studies of *trans*-1,2-Dichloroethylene[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	10% SP-1000 on 80/100 Supelcoport, 1.8 m × 4 mm	Nitrogen at 70 mL/minute	Isothermal at 50° C or 50° C for 5 minutes, then 10° C/minute to 250° C
System B			
Flame ionization	80/100 Porapak QS, 1.8 m × 4 mm	Nitrogen at 70 mL/minute	Isothermal at 200° C or 50° C for 5 minutes, then 10° C/minute to 225° C
System C			
Flame ionization	20% SP-2100/ 0.1% Carbowax 1500, 3.6 m × 2 mm	Nitrogen at 30 mL/minute	45° C for 18 minutes, then 20° C/minute to 165° C, held for 4 minutes
System D			
Mass spectrometry with full mass scan (70 eV; scan rate 1.05 seconds; multiplier voltage –1,700 or –1,800 V)	Megabore DB-624, 30 m × 0.53 mm, 3-µm film (J&W Scientific, Folsom, CA)	Helium at 10 mL/minute	30° C for 3 minutes, then 8° C/minute to 220° C
System E			
Mass spectrometry with selected ion monitoring (70 eV; scan rate 0.9 seconds for identification and 0.756 seconds for quantitation; multiplier voltage –2,000 V)	Megabore DB-624, 30 m × 0.53 mm, 3-µm film (J&W Scientific)	Helium at 10 mL/minute	30° C for 3 minutes, then 8° C/minute to 220° C
System F			
Flame ionization	20% SP-2100/ 0.1% Carbowax 1500, 3.6 m × 2 mm	Nitrogen at 30 mL/minute	45° C for 5 minutes, then 20° C/minute to 85° C, held for 2 minutes
System G			
Electron capture	DB-1 Megabore, 30 m × 0.53 mm, 3-µm film (J&W Scientific)	Nitrogen at approximately 9 mL/minute	Isothermal at 50° C

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

TABLE F2
Preparation and Storage of Dose Formulations in the 14-Week Feed Studies of *trans*-1,2-Dichloroethylene

Preparation
A premix of microencapsulated *trans*-1,2-dichloroethylene and feed was prepared by hand and then blended with additional feed in a twin-shell blender for 15 minutes, with the intensifier bar on for the first 5 minutes. The dose formulations were placed in a plastic bag, kneaded and mixed manually, then returned to the blender and mixed for an additional 15 minutes, with the intensifier bar on for the first 5 minutes. Dose formulations were prepared at least every 2 weeks.

Chemical Lot Number
Neat: MP-02224LP
Microcapsules: 343-1B-A

Maximum Storage Time
4 weeks

Storage Conditions
Stored in doubled plastic bags, protected from light, at 5° C

Study Laboratory
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 *trans*-1,2-Dichloroethylene

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
3 May 1993	5 May 1993[b]	3,125	3,870	+24
		6,250	7,510	+20
		12,500	14,600	+17
		25,000	29,600	+18
		50,000	59,500	+19
6 May 1993	7 May 1993[c]	3,125	3,180	+2
		6,250	5,970	-4
		12,500	12,900	+3
		25,000	24,200	-3
		50,000	47,700	-5
	21-22 June 1993[d]	3,125	2,860	-8
		6,250	4,530[e]	-28
		12,500	9,050[e]	-28
		25,000	19,400[e]	-22
		50,000	41,300	-17
11 May 1993	12 May 1993[b]	3,125	4,070	+30
		6,250	7,890	+26
		12,500	14,700	+18
		25,000	29,600	+18
		50,000	60,000	+20
14 May 1993	14 May 1993[c]	3,125	3,230	+3
		6,250	6,380	+2
		12,500	12,100	-3
		25,000	25,200	+1
		50,000	49,100	-2
17 May 1993	18 May 1993	3,125	3,390	+8
		6,250	6,480	+4
		12,500	13,300	+6
		25,000	25,700	+3
		50,000	52,700	+5
25 May 1993	1 June 1993	3,125	3,470	+11
		6,250	6,450	+3
		12,500	12,700	+2
		25,000	25,400	+2
		50,000	46,700	-7

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

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
22 June 1993	23 June 1993[b]	3,125	3,790	+21
		6,250	2,210	−65
		12,500	15,800	+26
		25,000	30,000	+20
		50,000	58,100	+16
29 June 1993	29 June 1993[c]	3,125	3,350	+7
		6,250	6,540	+5
		12,500	12,800	+2
		25,000	25,500	+2
		50,000	49,000	−2
	19 July 1993[d]	3,125	1,510	−52
		6,250	3,460	−45
		12,500	6,850	−45
		25,000	15,600	−38
		50,000	29,600	−41
16 July 1993	19 July 1993	3,125	2,590 [f]	−17
		6,250	5,320 [f]	−15
		12,500	11,800	−6
		25,000	24,900	0
		50,000	47,300	−5
22 July 1993	22 July 1993	3,125	2,980[g]	−5
		6,250	5,860[g]	−6
	28 September 1993[d]	3,125	1,900	−39
		6,250	3,960	−37
		12,500	6,640	−47
		25,000	14,700	−41
		50,000	29,100	−42
Mice				
3 May 1993	5 May 1993[b]	3,125	3,870	+24
		6,250	7,510	+20
		12,500	14,600	+17
		25,000	29,600	+18
		50,000	59,500	+19

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

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
6 May 1993	7 May 1993[c]	3,125	3,180	+2
		6,250	5,970	−4
		12,500	12,900	+3
		25,000	24,200	−3
		50,000	47,700	−5
	23 June 1993[d]	3,125	1,230	−61
		6,250	7,410	+19
		12,500	5,300	−58
		25,000	7,440	−70
		50,000	21,800	−56
	24 June 1993[h]	3,125	1,240	−60
		6,250	2,130	−66
		12,500	5,460	−56
		25,000	7,340	−71
		50,000	20,700	−59
11 May 1993	12 May 1993[b]	3,125	4,070	+30
		6,250	7,890	+26
		12,500	14,700	+18
		25,000	29,600	+18
		50,000	60,000	+20
14 May 1993	14 May 1993[c]	3,125	3,230	+3
		6,250	6,380	+2
		12,500	12,100	−3
		25,000	25,200	+1
		50,000	49,100	−2
17 May 1993	18 May 1993	3,125	3,390	+8
		6,250	6,480	+4
		12,500	13,300	+6
		25,000	25,700	+3
		50,000	52,700	+5
25 May 1993	1 June 1993	3,125	3,470	+11
		6,250	6,450	+3
		12,500	12,700	+2
		25,000	25,400	+2
		50,000	46,700	−7
22 June 1993	23 June 1993[b]	3,125	3,790	+21
		6,250	2,210	−65
		12,500	15,800	+26
		25,000	30,000	+20
		50,000	58,100	+16

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

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
29 June 1993	29 June 1993[c]	3,125	3,350	+7
		6,250	6,540	+5
		12,500	12,800	+2
		25,000	25,500	+2
		50,000	49,000	−2
	19 July 1993[d]	3,125	953	−70
		6,250	2,170	−65
		12,500	4,690	−62
		25,000	11,000	−56
		50,000	12,500	−75
16 July 1993	19 July 1993	3,125	2,590 [f]	−17
		6,250	5,320 [f]	−15
		12,500	11,800	−6
		25,000	24,900	0
		50,000	47,300	−5
22 July 1993	22 July 1993	3,125	2,980[g]	−5
		6,250	5,860[g]	−6
	28 September 1993[d]	3,125	1,550	−50
		6,250	3,470	−44
		12,500	5,900	−53
		25,000	12,300	−51
		50,000	17,800	−64

[a] Results of duplicate analyses
[b] Reblended with additional feed and reanalyzed
[c] Results for reblended samples
[d] Animal room samples
[e] Results of triplicate analyses
[f] Remixed; not used in study
[g] Results of remix
[h] Reanalysis of animal room samples