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

NTP Technical Report on the Toxicity Studies of 2,4-Decadienal (CAS No. 25152-84-5) Administered by Gavage to F344/N Rats and B6C3F$_1$ Mice

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National Toxicology Program
Toxicity Report Series
Number 76

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National Institutes of Health
Public Health Service
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FOREWORD

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Administered by Gavage
to F344/N Rats and B6C3F1 Mice

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PEER REVIEW

The draft report on the toxicity studies of 2,4-decadienal 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 this Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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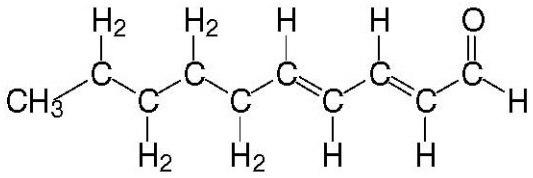
ABSTRACT



2,4-DECADIENAL

CAS No. 25152-84-5

Chemical Formula: $C_{10}H_{16}O$ Molecular Weight: 152.24

Synonyms: 2,4-De; deca-2,4-dienal; *trans,trans*-2,4-decadienal; *trans,trans*-2,4-decadien-1-al; heptenyl acrolein; RIFM#77-102

2,4-Decadienal is used as a synthetic flavoring and fragrance material and has been evaluated as a corrosion inhibitor for steel in oil field operations. 2,4-Decadienal was nominated by the National Cancer Institute for toxicity testing because the dienaldehydes occur naturally in a variety of foods and food components, are used as food additive/flavoring agents, and the potential for human exposure is high. In the toxicity studies, male and female F344/N rats and B6C3F1 mice received 2,4-decadienal (at least 93% pure) in corn oil by gavage for 2 weeks or 3 months. Genetic toxicology studies were conducted in *Salmonella typhimurium*, rat and mouse bone marrow cells, and mouse peripheral blood erythrocytes.

In the 2-week studies, groups of five male and five female rats and mice received 2,4-decadienal in corn oil by gavage at doses of 0, 45, 133, 400, 1,200, or 3,600 mg 2,4-decadienal/kg body weight 5 days per week for 16 days. All animals in the 3,600 mg/kg groups were found dead or sacrificed moribund by day 3 (rats) or day 9 (mice). One 133 mg/kg female rat was found dead on day 8, and one male and one female mouse in the 1,200 mg/kg groups were found dead on days 12 and 16, respectively. At 1,200 mg/kg, treatment-related ulceration of the forestomach was observed in male and female rats and mice. Focal necrosis of the forestomach occurred in a 1,200 mg/kg female mouse. Mean body weights of all 1,200 mg/kg groups were less than those of the vehicle controls, and 1,200 mg/kg female mice lost weight during the study. Diarrhea, lethargy, abnormal breathing (rats), and thinness (mice) occurred

in the 1,200 and 3,600 mg/kg groups. Gross lesions seen at necropsy included ulcerations of the forestomach in 1,200 mg/kg rats and 1,200 and 3,600 mg/kg mice. Adhesions involving the stomach and other abdominal organs were also seen in 1,200 and 3,600 mg/kg mice.

In the 3-month studies, groups of 10 male and 10 female rats and mice received 2,4-decadienal in corn oil by gavage at doses of 0, 50, 100, 200, 400, or 800 mg 2,4-decadienal/kg 5 days per week for 14 weeks. No chemical-related deaths occurred. Mean body weights of 400 mg/kg male rats and 800 mg/kg male and female rats and male mice were significantly less than those of the vehicle controls. Dosed male and female rats were lethargic after week 7; the severity of the lethargy was dose related. There were changes in the leukon of dosed rats compared to vehicle control rats characterized by decreased leukocyte, lymphocyte, and eosinophil counts and increased neutrophil counts. Spleen weights of 800 mg/kg female rats and thymus weights of 400 and 800 mg/kg female rats were significantly less than those of the vehicle controls. Thymus, spleen, testis, cauda epididymis, and epididymis weights of 800 mg/kg male rats were less than those of the vehicle controls.

The incidences of epithelial hyperplasia of the forestomach were significantly greater in 400 and 800 mg/kg male and female rats, 200, 400, and 800 mg/kg male mice, and 800 mg/kg female mice than in the vehicle controls. Incidences of epithelial degeneration of the forestomach were significantly increased in 800 mg/kg rats and the incidence of chronic active inflammation of the forestomach was significantly increased in 800 mg/kg female rats. Incidences of exudate and olfactory epithelial atrophy of the nose were significantly increased in 800 mg/kg male rats, and incidences of olfactory epithelial necrosis occurred in 200 mg/kg or greater mice. Olfactory epithelial hydropic degeneration occurred in a single female mouse from the 100 mg/kg group.

2,4-Decadienal was not mutagenic in any of several strains of *S. typhimurium* tested with and without liver S9 activation enzymes. Acute bone marrow micronucleus tests in laboratory rodents administered 2,4-decadienal by intraperitoneal injection yielded mixed results. In male rats, a single injection of 2,4-decadienal gave a positive response, but no confirmatory trial was conducted. In male mice, a standard three-injection bone marrow micronucleus experiment yielded negative results but a 48-hour bone marrow analysis after a single dose of 600 mg/kg revealed a small but statistically significant increase in micronucleated polychromatic erythrocytes. Analysis of peripheral blood erythrocytes in these same mice also showed a dose-related increase in micronucleated polychromatic cells, but the increase was insufficient for a positive call and the results of the acute micronucleus assays in mice were judged to be equivocal overall. No increase in the frequency of micronucleated normochromatic erythrocytes was seen in peripheral blood of male or female mice administered 2,4-decadienal by gavage for 3 months.

In summary, 2,4-decadienal administration caused decreased body weights and increased incidences of forestomach lesions in the 3-month studies in rats and mice. In addition, treatment-related lesions of the olfactory epithelium were observed in male rats and male and female mice. The no-observed-adverse-effect level was determined to be 100 mg/kg in rats and mice. 2,4-Decadienal was not mutagenic *in vitro* or *in vivo*.

INTRODUCTION

2,4-Decadienal is a lipid peroxidation product found in numerous meat, vegetable, and fish oils. Polyunsaturated oils are susceptible to auto-oxidation during storage (Snyder *et al*., 1985), and auto-oxidation products have been implicated in the development of off or tainted flavor. Several researchers have implied there could be a link between exposures to lipid peroxidation products in the diet and the development of human cancers. Lipid hydroperoxides have been shown to give rise to low intracellular levels of 2,4-decadienal and other α,β-unsaturated aldehydes that are known to be reactive with DNA (Frankel *et al*., 1987). Ingested lipid oxidation products and oxidized fats have been reported to cause increased excretion of mutagens, cellular injury to liver and kidneys, increased cell proliferation in the gastrointestinal tract, and other nonspecific tissue injury and irritation effects resulting from induced oxidative stress.

Chemical and Physical Properties

2,4-Decadienal is a colorless to yellowish liquid with a fresh, grassy odor. It has a boiling point of 58° to 61° C at 0.05 mm Hg and 114° to 116° C at 10 mm Hg, a flash point of 101° C, a vapor density greater than 1.0, a vapor pressure of 0.02 mm Hg, and a specific gravity of 0.857 at 20° C (Aldrich, 1992; *Heilbron's*, 1992). 2,4-Decadienal is soluble in water (Aikawa and Chikuni, 1988).

Production, Use, and Human Exposure

2,4-Decadienal is produced by the condensation of hexaldehyde with crotonaldehyde (Opdyke, 1979) or by the oxidation of linoleic acid (*Heilbron's*, 1992). Although current production rates are not available, the Food and Drug Administration's Priority-based Assessment of Food Additives database reported a market disappearance rate of 80 pounds per year for the survey year 1987 (USFDA, 1992). Production of up to 1,000 pounds per year has been reported for use in fragrances (Opdyke, 1979). 2,4-Decadienal is used as a synthetic flavoring and fragrance material (Opdyke, 1979; *Heilbron's*, 1992) and has been evaluated as a corrosion inhibitor for steel in oil field operations (Growcock *et al.*, 1989).

2,4-Decadienal is found as a contaminant in water (Bao *et al.,*1997). It is generated from polyunsaturated fatty acids by the action of plant lipoxygenases (Almosnino and Belin, 1991; Andrianarison *et al.,* 1991) and is produced in mammalian tissues in certain physiological and pathophysiological processes such as lipid peroxidation, arachidonic

acid oxidation, and reactions with reactive oxygen species (Esterbauer, 1985). In addition, scores of foods including fruit, vegetable, meat, and processed food products contain 2,4-decadienal either naturally or as an additive or flavoring agent, making low-level human exposure virtually universal. Feron *et al.* (1991) reported that 2,4-decadienal had been identified in 80 foods, with the highest concentration, 500 ppm, detected in the oil of tangerine peel. The presence of 2,4-decadienal in canned asparagus and meat products has been related to odor and spoilage. Other processed foods in which it has been found include mushrooms, salted and pickled prunes, and frozen strawberries (Josephson and Lindsay, 1987; Grun *et al.,* 1996; Milo and Grosch, 1996). 2,4-Decadienal is also present in oxidized, heated, or cooked edible animal and vegetable fats and oils, including heated and off-flavor or rancid butter and rancid peanut butter.

2,4-Decadienal has been listed as one of the most important flavor components of canola, corn, cottonseed, olive, peanut, safflower, soybean, and sunflower oils, and its concentration generally increases during storage (Snyder *et al.*, 1985). It is also in the essential oils of brown algae and *Patrina scarba*, a traditional Chinese drug; dried and stored Japanese piled tea; Indian black teas; roasted Colombian coffee beans; ginseng oil; and the essential oils of gentian and cinchona bark used in the production of liqueurs. It is an aroma volatile that has been associated with the off flavor of packaged, stored green tea, but the analog 2,4-heptadienal was found to be more typical of green tea. 2,4-Decadienal has also been identified in the flavor volatiles of tonka beans, which are not used in foods. Tokarska *et al.* (1986) reported that 2,4-decadienal was not found in fresh canola oil samples but reached concentrations exceeding 17 ppm following 16 weeks of storage. In these studies, rapid increases of 2,4-decadienal concentrations (exceeding tenfold) were seen in oil stored for 16 weeks in clear glass bottles, both with and without the addition of butylated hydroxytoluene/butylated hydroxyanisole/citric acid antioxidants at the maximum allowable concentration of 200 ppm (Canadian limit). Addition of 200 ppm *t*-butylhydroquinone as an antioxidant reduced 2,4-decadienal concentrations to 1.7 to 3.5 ppm; oxidative changes were also reduced by 100 ppm *t*-butylhydroquinone. Storage in amber bottles helped deter degradative changes during the first 12 weeks of storage, after which the occurrence of off-flavor volatiles began to increase more markedly. In another study, the concentrations of *trans,trans*-2,4-decadienal in unhydrogenated soybean oil as well as in aged, processed soybean and corn oils correlated with flavor panel scores as indicators of flavor quality (Raghavan *et al*., 1989).

2,4-Decadienal is a secondary degradation product of 9-hydroperoxylinoleic acid from oxidative deterioration of the polyunsaturated fatty acid linoleic acid (Katsuki *et al.,* 1987); these authors reported that the compound is one of the three major oxidation products of linoleic acid, along with pentane and hexanal. During auto-oxidation of linoleate at moderate temperatures (below 75° C), 2,4-decadienal appears as a result of cleavage of the linolyl residue between carbons eight and nine; as the temperature rises (to 180° C), 2,4-decadienal is formed in increasing quantity (Grosch, 1987). This author also reported that 2,4-decadienal is also a major volatile carbonyl compound found in the

auto-oxidation of arachidonic acid at 20° C, and it may be attacked by peroxy radicals to form peroxides that decompose to hexanal. Due to its reactivity, 2,4-decadienal is susceptible to further degradation via auto-oxidation, hydration, and retro-aldol condensation reactions (Josephson and Lindsay, 1987; Josephson and Glinka, 1989). Grein *et al*. (1993) demonstrated that water-mediated oxidative decomposition of 2,4-decadienal yielded hexanal, hexanoic acid, 2-octenal, 2-octenoic acid, 1-(2′-furyl)-hexan-1-ol, 6-hydroxy-2,4-decadienal, *trans*-4,5-epoxy-2-decenal, 4-hydroxy-2-nonenal, 1-pentanol, and 2-nonen-4-olide.

In addition to unhydrogenated, oxidized, heated, or cooked vegetable oils, 2,4-decadienal is also associated with meat-derived fats and oils, including warmed-over beef flavored volatiles, uncured pork, cooked beef, lamb, chicken, stored ground beef patties, turkey breakfast sausage, oxidized menhaden oil, and mussels (Snyder *et al.*, 1985). The oxidation of unsaturated fatty acids yields carbonyl compounds that contribute significantly to the flavor of uncured but not cured meat (Ramarathnan *et al.*, 1991). These investigators reported a concentration of 0.69 mg 2,4-decadienal/kg meat in uncured pork but the compound was not detected in cured pork.

To better understand the chemical nature of the flavor degradation of deep-fried chicken or fish held for 3 days at 6° C, Josephson and Lindsay (1987) studied the further degradation of 2,4-alkadienals using 2,4-decadienal in a water-mediated retro-aldol condensation reaction as a model system. 2,4-Decadienal degraded to 2-octenal and ethanal, followed by degradation of the 2-octenal to hexanal and ethanal at a rate independent of oxygen but greatly accelerated by heat. Two other compounds that were formed in significant quantities were tentatively identified as either *cis*- and *trans*-3-keto-4-decenals or *anti*- and *syn*-2-carboxyaldehyde-5-pentyl-2,5-dihydrofurans. In a study simulating deep-frying interactions, 42 and 45 volatile compounds were identified from reaction of 2,4-decadienal with the sulfur-containing natural food materials glutathione and cysteine, respectively (Zhang and Ho, 1989).

2,4-Decadienal was given Generally Recognized as Safe status after a review of flavoring ingredients and food additives by the Flavoring Extract Manufacturers' Association and was listed in 1974 by the Council of Europe as a flavoring substance that may be added to food (Opdyke, 1979). 2,4-Decadienal is listed in the U.S. Environmental Protection Agency's Toxic Substances Control Act Inventory (USEPA, 2003). No standards or guidelines have been set for occupational exposures or environmental concentrations of 2,4-decadienal.

METABOLISM

Experimental Animals

Aldehydes are principally metabolized by NADP-dependent aldehyde dehydrogenase to the corresponding acids (Weiner, 1982) or by alcohol dehydrogenase and aldehyde reductase to alcohols (Brophy and Barrett, 1990). NADP-dependent aldehyde dehydrogenase is ubiquitous in every organ system. A flavin-dependent aldehyde oxidase has also been shown but its importance is not known (Weiner, 1982). Exogenous 2,4-decadienal is probably metabolized to its acid in the liver.

The β-carbon of unsaturated aldehydes is a prime target for soft electrophiles like glutathione or cysteine. Conjugation of glutathione with lipid peroxidation-derived 2,4-decadienal catalyzed by endogenous glutathione-*S*-transferase from lean pork muscle (Williamson and Ball, 1988) and of *trans,trans*-2,4-decadienal catalyzed by glutathione-*S*-transferase from lamb muscle (Williamson, 1989) has been reported. Accordingly, conjugation has been proposed as a secondary mechanism for 2,4-alkadienal detoxification (Brophy and Barrett, 1990); these authors reported that a cytosolic glutathione transferase isolated from a mouse fibroblast cell line reduced secondary products of lipid peroxidation including *trans,trans*-2,4-decadienal. Grein *et al*. (1993) reported that in water-mediated oxidative decomposition of 2,4-decadienal, the predominant products were hexanal, hexanoic acids, and 4-hydroxy-2-nonenal.

Humans

No information on the metabolism of 2,4-decadienal in humans was found in the literature.

TOXICITY

Experimental Animals

The oral LD_{50} for 2,4-decadienal is greater than 5 g/kg body weight for rats and the dermal LD_{50} is 1.25 to 2.5 g/kg in rabbits; 2,4-decadienal causes moderate to severe irritation when applied dermally to rabbits (Opdyke, 1979; RTECS, 1992).

2,4-Decadienal is a strong electrophile and it reacts readily with nucleophilic groups such as nuclear DNA; very little information is available, however, on its effects *in vivo*. 2,4-Decadienal exhibits cytotoxic effects including inhibition of cell growth, alteration of cellular viability and glutathione concentration, and promotion of DNA fragmentation (Nappez *et al*., 1996). Part of the cytotoxic effect of 2,4-decadienal can be related to its ability to react with cellular nucleophiles (nucleic acids and proteins). Carvalho *et al*. (2000) demonstrated that 2,4-decadienal reacted with 2′-deoxyadenosine to form adducts. 2,4-Decadienal also reacted with 2′-deoxyguanosine in the

presence of peroxides to form adducts (Loureiro *et al*., 2000). The formation of adducts between nucleosides and secondary lipid oxidation products has been postulated as one of the mechanisms involved in the carcinogenic action of reactive breakdown compounds of lipid peroxides; however, carcinogenic activity of 2,4-decadienal has not been demonstrated.

Humans

Health hazard advisory information listed in the Material Safety Data Sheet for 2,4-decadienal states that the chemical is harmful if inhaled or swallowed and that it may cause irritation (Aldrich, 1992). A 48-hour closed-patch irritation test in humans with 5% 2,4-decadienal in petrolatum produced no irritation; the same concentration in a maximization test for sensitization produced no sensitization reaction (Opdyke, 1979). Kaneko *et al.* (1987) studied linoleic acid and some of its aldehydic auto-oxidation products for toxicity to human diploid fibroblasts. Following 1-day exposures, 2,4-decadienal was observed to be the most toxic and to show similar toxicity to both proliferating and arrested cells. 2,4-Decadienal was highly cytotoxic to cultured human endothelial cells, with an LC_{50} of 9 µM (Kaneko *et al.*, 1988).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No reproductive or developmental toxicity studies of 2,4-decadienal in experimental animals or humans were found in a review of the literature.

CARCINOGENICITY

Experimental Animals

No information on the carcinogenicity of 2,4-decadienal in experimental animals was found in a review of the literature. However, α,β-unsaturated carbonyl compounds such as malondialdehyde (Siu *et al*., 1983), 3,4,5-trimethoxycinnamaldehyde (Schoental and Gibbard, 1972), crotonaldehyde (Chung *et al*., 1986), and 2-chloro- and 2-bromoacrolein (Robinson *et al*., 1989) have been shown to be carcinogenic. A related chemical, 2,4-hexadienal, administered by gavage, was carcinogenic, inducing forestomach neoplasms in male and female rats and mice (Chan *et al*., 2003; NTP, 2003).

Humans

No epidemiology studies or case reports associating 2,4-decadienal exposure with cancer risk in humans were found in the literature.

Genetic Toxicity

There are no published data for 2,4-decadienal tested in classic mutagenicity assays. When tested in *Escherichia coli* strain B/r WP2, 2,4-decadienal (50 to 200 μg/plate) reduced the number of *trp*$^{+}$ revertants induced by exposure to UV light in a dose-related manner (Aikawa and Chikuni, 1988). The authors indicated that additional tests had shown that the reduction in mutant *E. coli* colonies seen in the presence of 2,4-decadienal was not due to selective toxicity but rather to antimutagenic activity. In contrast to this single report of apparent antimutagenic activity, 2,4-decadienal was shown to form adducts with free 2′-deoxyadenosine after an epoxidation reaction (Carvalho *et al*., 1998) and the authors suggested that similar endogenous reactions with 2,4-decadienal might contribute to *in vivo* genotoxicity and cytotoxicity in mammalian cells.

Study Rationale and Design

2,4-Decadienal was nominated, together with 2,4-hexadienal, by the National Cancer Institute for toxicity testing because the dienaldehydes occur naturally in a variety of foods and food components and are used as food additive/flavoring agents and the potential for human exposure is high.

This Toxicity Study Report describes the results of 2-week and 3-month toxicity studies in which 2,4-decadienal was administered in corn oil by gavage to rats and mice. Gavage was chosen as the route of exposure because the oral route is the most likely exposure route for humans and these compounds are unstable when mixed in feed preparations and insoluble in water. The doses chosen were 0, 45, 133, 400, 1,200, and 3,600 mg 2,4-decadienal/kg body weight per day in the 2-week studies and 0, 50, 100, 200, 400, and 800 mg/kg per day in the 3-month studies in rats and mice.

MATERIALS AND METHODS

Procurement and Characterization of 2,4-Decadienal

2,4-Decadienal was obtained from Lancaster Synthesis (Windham, NH) in one lot (90000694). Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC) and the study laboratories, Microbiological Associates, Inc. (Bethesda, MD), and Southern Research Institute (Birmingham, AL) (Appendix F). Reports on analyses performed in support of the 2,4-decadienal studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a pale yellow liquid, was identified as 2,4-decadienal by infrared and nuclear magnetic resonance spectroscopy. The purity of lot 90000694 was determined by gas chromatography. Gas chromatography by two systems indicated one major peak and six impurities. The overall purity was determined to be at least 93%.

To ensure stability, the bulk chemical was stored at approximately 5° C in the shipping container under nitrogen. Stability was monitored during the 2-week and 3-month studies using gas chromatography. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared once during the 2-week studies and every 3 to 4 weeks during the 3-month studies by mixing 2,4-decadienal with corn oil (Table F1). Homogeneity studies of 5 and 160 mg/mL dose formulations and stability studies of a 4.5 mg/mL dose formulation were performed by the analytical chemistry laboratory using high performance liquid chromatography (HPLC). Homogeneity was confirmed, and stability was confirmed for at least 35 days for dose formulations stored at refrigerator (2° to 5° C) or room (23° to 28° C) temperature in sealed glass vials.

Periodic analyses of the dose formulations of 2,4-decadienal were conducted at the study laboratory using HPLC. During the 2-week studies, the dose formulations were analyzed once (Table F2). All five of the dose formulations used in the 2-week studies for each species were within 10% of the target concentration. During the 3-month studies, the dose formulations were analyzed three times (Table F3). All 15 of the dose formulations used in the 3-month studies for each species were within 10% of the target concentration.

2-Week Studies

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 11 (rats) or 12 (mice) days and were 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice received 2,4-decadienal in corn oil by gavage at doses of 0, 45, 133, 400, 1,200, or 3,600 mg 2,4-decadienal/kg body weight 5 days per week for 16 days. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. The animals were weighed and clinical findings were recorded initially, on day 8, and at the end of the studies. Before the studies began, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease.

Necropsies were performed on all rats and mice. The right kidney and liver were weighed. Histopathologic examinations were performed on vehicle control, 400, 1,200, and 3,600 mg/kg rats and mice. Table 1 lists the tissues and organs examined.

3-Month Studies

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 11 to 14 days and were 6 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 was collected from five male and five female rats and mice at the end of the 3-month studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results were negative.

Groups of 10 male and 10 female rats and mice received 2,4-decadienal in corn oil by gavage at doses of 0, 50, 100, 200, 400, or 800 mg/kg 5 days per week for 14 weeks. Additional groups of 10 male and 10 female rats designated for clinical pathology testing were administered the same doses for up to 19 days. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Core study animals were weighed initially, on day 4 (rats), weekly, and at the end of the studies. Clinical findings were recorded initially (mice), on day 4 (core study rats), weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 19 and from all core study rats and mice at the end of the studies for hematology and clinical chemistry (rats) analyses. The animals were anesthetized with a mixture of carbon dioxide and oxygen. Samples for hematology analyses were placed into tubes containing EDTA; samples for clinical chemistry evaluations were placed in similar tubes without anticoagulant.

Hematology and clinical chemistry blood samples were maintained at room temperature and transported in carrying containers to the clinical pathology laboratory after collection. Hematocrit; hemoglobin concentration; erythrocyte, platelet, and leukocyte counts; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined with a Technicon H·1® (Technicon Corporation, Tarrytown, NY). Reticulocyte counts were conducted using a Coulter Model Elite Flow Cytometer (Coulter Corporation, Miami, FL). Differential leukocyte counts were determined by light microscopy from blood smears. For clinical chemistry analyses, serum samples were analyzed using a Roche Cobas Fara automated analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). Hematology and clinical chemistry analyses were performed using commercially available reagents. The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm count and motility and vaginal cytology evaluations on core study rats and mice administered 0, 200, 400, or 800 mg/kg. The parameters evaluated are listed in Table 1. 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, spleen, 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 the vehicle control and 800 mg/kg core study rats and mice. The forestomach and nose were examined in all remaining groups of rats and mice, and the glandular stomach was examined in 100, 200, and 400 mg/kg male mice and 200 and 400 mg/kg female mice. Table 1 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 1
Experimental Design and Materials and Methods in the Gavage Studies of 2,4-Decadienal

2-Week Studies	3-Month Studies
Study Laboratory	
Microbiological Associates, Inc. (Bethesda, MD)	Southern Research Institute (Birmingham, AL)
Strain and Species	
F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 11 days Mice: 12 days	Rats: 11 days (males) or 12 days (females) Mice: 13 days (males) or 14 days (females)
Average Age When Studies Began	
6 weeks	6 weeks
Date of First Dose	
Rats: November 13, 1995 Mice: November 14, 1995	Rats: July 29 (males) or 30 (females), 1996 Mice: July 31 (males) or August 1 (females), 1996
Duration of Dosing	
5 days/week for 16 days	5 days/week for 14 weeks
Date of Last Dose	
Rats: November 28, 1995 Mice: November 29, 1995	Rats: October 30 (males) or 31 (females), 1996 Mice: November 1 (males) or 2 (females), 1996
Necropsy Dates	
Rats: November 29, 1995 Mice: November 30, 1995	Rats: October 30 (males) or 31 (females), 1996 Mice: November 1 (males) or 2 (females), 1996
Average Age at Necropsy	
Rats: 9 weeks Mice: 8 weeks	Rats: 19 weeks Mice: 20 weeks
Size of Study Groups	
5 males and 5 females	10 males and 10 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies
Animals per Cage	
Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 5 Mice: 1 (males) or 5 (females)

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of 2,4-Decadienal

2-Week Studies	3-Month Studies
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
NTP-2000 pelleted feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 2-week studies, except irradiated
Water	
Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system, available *ad libitum*	Tap water (Birmingham municipal supply) via automatic watering system (Edstrom Industries, Inc.), available *ad libitum*
Cages	
Solid-bottom polycarbonate (Lab Products, Inc., Maywood, NJ), changed once (male mice) or twice (rats and female mice) weekly	Same as 2-week studies
Bedding	
Heat-treated hardwood chips (P.J. Murphy Forest Products, Inc., Montville, NJ), changed once (male mice) or twice (rats and female mice) weekly	Same as 2-week studies, except irradiated
Cage Filters	
None	Reemay® spun-bonded polyester filters (Andico, Birmingham, AL), changed biweekly
Racks	
Stainless steel, rotated biweekly	Same as 2-week studies
Animal Room Environment	
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses	
0, 45, 133, 400, 1,200, 3,600 mg/kg in corn oil; dosing volume = 2.5 mL/kg (0 to 1,200 mg/kg groups) or 5.0 mL/kg (3,600 mg/kg group)	0, 50, 100, 200, 400, or 800 mg/kg in corn oil; dosing volume = 5 mL/kg (rats) or 10 mL/kg (mice)
Type and Frequency of Observation	
Observed twice daily; animals were weighed and clinical findings were recorded initially, on day 8, and at the end of the studies.	Observed twice daily; core study rats and mice were weighed initially, on day 4 (rats), weekly, and at the end of the studies; clinical findings were recorded initially (mice), on day 4 (core study rats), weekly, and at the end of the studies.
Method of Sacrifice	
Carbon dioxide asphyxiation	Carbon dioxide asphyxiation
Necropsy	
Necropsies were performed on all animals. Organs weighed were the right kidney and liver.	Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, spleen, right testis, and thymus.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of 2,4-Decadienal

2-Week Studies	3-Month Studies
Clinical Pathology	
None	Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 19 and from all core study rats and mice at the end of the studies for hematology and clinical chemistry (rats) determinations. *Hematology*: automated hematocrit; hemoglobin concentration, erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin, mean cell hemoglobin concentration, and leukocyte counts and differentials *Clinical chemistry*: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids
Histopathology	
In addition to gross lesions and tissue masses, the kidney, liver, and forestomach from vehicle control, 400 (forestomach only), 1,200, and 3,600 mg/kg rats and mice were examined.	Complete histopathology was performed on vehicle control and 800 mg/kg rats and mice. 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 (except male mice), nose, ovary, pancreas, pancreatic islets, 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 (rats only), and uterus. The forestomach and nose were examined in all remaining rat and mouse groups, and the glandular stomach was examined in 100 (males), 200, and 400 mg/kg mice.
Sperm Count and Motility and Vaginal Cytology	
None	At the end of the studies, sperm samples were collected from male animals in the 0, 200, 400, and 800 mg/kg groups for sperm count and 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 prior to the end of the studies from females exposed to 0, 200, 400, or 800 mg/kg 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 dosed and vehicle control groups in the analysis of continuous variables. Organ and body weight data, which historically 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), as modified by Williams (1986), 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 (1957) 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 doses.

QUALITY ASSURANCE METHODS

The 3-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Unit of Southern Research Institute performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1992). 2,4-Decadienal was sent to the laboratories as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, TA102, TA104, and TA1535 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 2,4-decadienal. The high dose was limited by toxicity. All trials conducted without S9 were repeated and trials initially conducted with 10% S9 were repeated with 30% S9 in the culture.

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.

Rat and Mouse Bone Marrow and Peripheral Blood Micronucleus Test Protocols

Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by 2,4-decadienal exposure. The standard three-exposure protocol (three intraperitoneal injections at 24-hour intervals) is described in detail by Shelby *et al.* (1993); the protocol used in the single-injection studies is similar except only one injection was administered. Male F344/N rats were injected once and male B6C3F1 mice were injected once or three times with 2,4-decadienal dissolved in corn oil. Vehicle control animals were injected with corn oil only. The positive control animals received injections of cyclophosphamide. In the single injection studies, animals were killed 24 (rats) or 48 (mice) hours after injection; in the three-exposure study, mice were killed 24 hours after the third injection. Blood smears were prepared from bone marrow cells obtained from the femurs in all studies and peripheral blood samples collected in the single injection mouse study. Air-dried smears of bone marrow and peripheral blood were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of four or five animals per dose group.

At the end of the 3-month study, peripheral blood samples were obtained from male and female mice. A detailed discussion of this assay is presented by MacGregor *et al.* (1990). Smears were immediately prepared, fixed, stained, and coded as described for the intraperitoneal injection studies. Slides were scanned to determine the frequency of micronuclei in 1,000 normochromatic erythrocytes (NCEs) in each of nine or 10 animals per dose group. Results of the 3-month study were accepted without repeat tests, because additional test data could not be obtained.

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 PCEs or NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (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 dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

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

2-WEEK STUDY

All 3,600 mg/kg rats were found dead or sacrificed moribund by day 3, and one 133 mg/kg female was found dead on day 8 (Table 2). Final mean body weights and body weight gains of 1,200 mg/kg males and females were significantly less than those of the vehicle controls. Diarrhea, lethargy, and abnormal breathing occurred in 1,200 and 3,600 mg/kg rats.

Organ weight differences between the 1,200 mg/kg groups and the vehicle controls were related to decreased body weights (Table C1). Gross lesions seen at necropsy included ulcerations of the forestomach in all male and four female 1,200 mg/kg rats. Treatment-related ulceration of the forestomach was observed microscopically at 3,600 mg/kg in all rats and at 1,200 mg/kg in all male and the majority of female rats. Forestomach ulceration in both sexes at 3,600 mg/kg was of sufficient severity to account for morbidity and mortality in these animals.

Dose Selection Rationale: Due to high mortality in the 3,600 mg/kg groups and forestomach lesions in the 1,200 and 3,600 mg/kg groups, doses selected for the 3-month study in rats were 0, 50, 100, 200, 400, and 800 mg/kg.

TABLE 2
Survival and Body Weights of Rats in the 2-Week Gavage Study of 2,4-Decadienal

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	97 ± 5	179 ± 5	81 ± 2	
45	5/5	99 ± 5	184 ± 7	86 ± 2	103
133	5/5	96 ± 4	176 ± 4	81 ± 2	99
400	5/5	95 ± 5	168 ± 6	73 ± 2	94
1,200	5/5	97 ± 6	140 ± 10**	43 ± 4**	78
3,600	0/5[c]	95 ± 3	—	—	—
Female					
0	5/5	88 ± 4	130 ± 4	42 ± 1	
45	5/5	86 ± 6	131 ± 6	45 ± 2	101
133	4/5[d]	88 ± 4	131 ± 6	43 ± 1	101
400	5/5	87 ± 5	135 ± 4	47 ± 2	104
1,200	5/5	86 ± 4	110 ± 5*	24 ± 4**	85
3,600	0/5[c]	86 ± 5	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' or Dunnett's test
[a] Number of animals surviving at 17 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Day of death: 2, 2, 3, 3, 3
[d] Day of death: 8

3-Month Study

All rats survived to the end of the study (Table 3). Final mean body weights and body weight gains of 400 and 800 mg/kg males and 800 mg/kg females were significantly less than those of the vehicle controls (Table 3 and Figure 1). Salivation occurred in all dosed males and females. Males and females administered 200 mg/kg or greater were lethargic after dosing beginning week 7; rats in the 50 and 100 mg/kg dose groups were occasionally lethargic after week 7.

Table 3
Survival and Body Weights of Rats in the 3-Month Gavage Study of 2,4-Decadienal

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	106 ± 3	329 ± 6	223 ± 5	
50	10/10	104 ± 2	332 ± 10	229 ± 8	101
100	10/10	103 ± 3	321 ± 6	217 ± 4	97
200	10/10	106 ± 3	313 ± 7	208 ± 6	95
400	10/10	104 ± 2	296 ± 5**	193 ± 4**	90
800	10/10	105 ± 3	265 ± 7**	160 ± 6**	81
Female					
0	10/10	101 ± 2	200 ± 3	100 ± 3	
50	10/10	100 ± 3	198 ± 4	97 ± 3	99
100	10/10	99 ± 2	194 ± 3	96 ± 2	97
200	10/10	100 ± 2	190 ± 2	90 ± 2	95
400	10/10	101 ± 1	195 ± 3	95 ± 2	98
800	10/10	100 ± 2	189 ± 3*	89 ± 2**	94

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
** Significantly different ($P \leq 0.01$) 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.

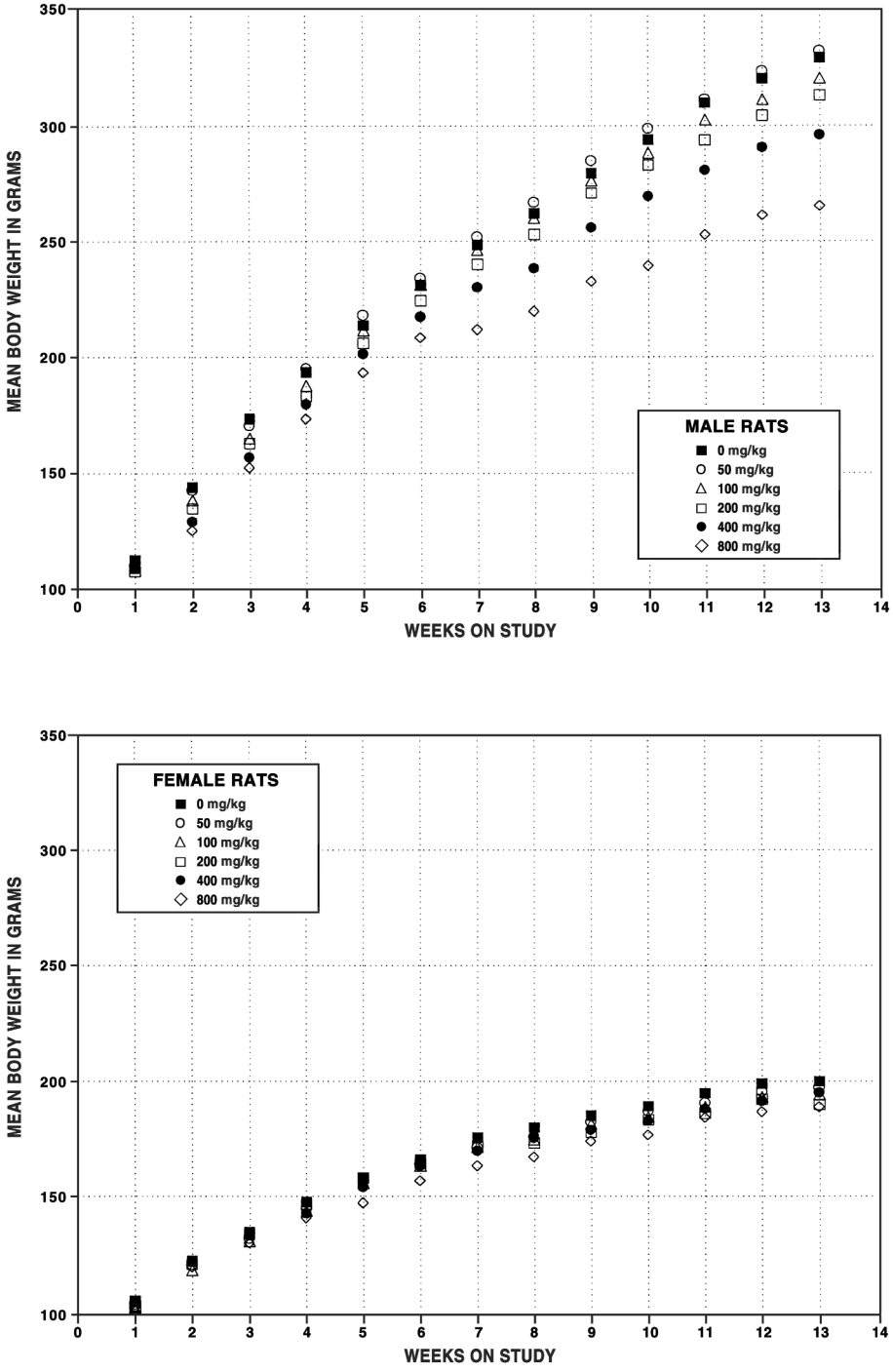


FIGURE 1
Body Weights of Male and Female Rats Administered 2,4-Decadienal by Gavage for 3 Months

The hematology and clinical chemistry data for rats in the 3-month toxicity study of 2,4-decadienal are listed in Table B1. There were changes in the leukon that, in general, would be consistent with a physiological stress/steroid-induced type response. The leukon alterations were characterized by decreases in leukocyte, lymphocyte, and eosinophil counts and increases in neutrophil counts. And, though the leukocyte response was most pronounced on day 4, it occurred, essentially, at all time points and in both sexes. At week 14, there were minimal (<10%) increases in hematocrit values, hemoglobin concentrations, and erythrocyte counts in the 800 mg/kg male and female rats. Although this change in the erythron was an enigma, it occurred only in the 800 mg/kg group, was of minimal severity, and would be consistent with a physiological response such as the hemoconcentration of dehydration. Other scattered changes in the hematological variables occurred but were not considered toxicologically relevant.

On day 4, there were increases in albumin, total protein, and blood urea nitrogen concentrations in dosed rats. This change was transient and would be consistent with a physiological dehydration-type response. Alkaline phosphatase activity was generally decreased in dosed rats throughout the study. It has been suggested that decreased alkaline phosphatase activity may be related to altered food intake (Imai *et al.*, 1991; Travlos *et al.*, 1996). In this study, there were treatment-related decreases in body weight and weight gain to suggest an altered nutritional state. Thus, the decreases in alkaline phosphatase activity were possibly related to a decreased food intake rather than a direct compound effect on enzyme activity. In a study using a rat CCl_4-induced hepatic model (Ferre *et al.*, 1999), higher concentrations of 2,4-dialkenals (2,4-heptadienal and 2,4-decadienal) were detected in rats and these higher concentrations coincided with a higher percentage of plasma alanine aminotransferase activity and hepatic fibrosis. The authors suggested that the increased 2,4-dialkenal concentrations may be related to the severity of hepatic injury in this model. In the current study, however, there were no increases in serum alanine aminotransferase or sorbitol dehydrogenase activities to indicate hepatocellular damage related to 2,4-decadienal treatment. Other scattered changes in the clinical chemistry variables occurred but were not considered toxicologically relevant.

Thymus weights in 800 mg/kg males and 400 and 800 mg/kg females were less than those of the vehicle controls (Table C2). Spleen weights of 800 mg/kg males and females were less than those of the vehicle controls. Testis, cauda epididymis, and epididymis weights of 800 mg/kg males were less than those of the vehicle controls (Table D1). The decreased organ weights were considered secondary to the decreased body weights.

No treatment-related gross lesions were observed at necropsy. In the forestomach of rats, there were significantly increased incidences of epithelial hyperplasia at 400 and 800 mg/kg and epithelial degeneration at 800 mg/kg (Tables 4, A1, and A2). The incidences of epithelial degeneration at 400 mg/kg, although not statistically significant, were higher than in the vehicle controls. One of the 800 mg/kg males had concurrent minimal, focal, duodenal erosion (Table A1). The incidences of chronic active inflammation of the forestomach in rats administered 400 or

TABLE 4
Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Gavage Study of 2,4-Decadienal

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Male						
Stomach, Forestomach[a]	10	10	10	10	10	10
Epithelium, Hyperplasia[b]	0	0	0	0	4* (1.0)[c]	6** (1.0)
Epithelium, Degeneration	0	0	0	0	1 (2.0)	5* (1.2)
Inflammation, Chronic Active	0	0	0	0	2 (1.0)	2 (1.0)
Nose	10	10	10	9	10	10
Exudate	0	0	0	0	2 (1.0)	6** (1.0)
Olfactory Epithelium, Atrophy	0	0	0	1 (2.0)	1 (3.0)	4* (1.5)
Olfactory Epithelium, Necrosis	0	0	0	1 (1.0)	0	0
Female						
Stomach, Forestomach	10	10	9	10	10	10
Epithelium, Hyperplasia	0	0	0	0	6** (1.0)	10** (1.0)
Epithelium, Degeneration	0	0	0	0	2 (1.0)	10** (1.3)
Inflammation, Chronic Active	0	1 (1.0)	0	0	2 (1.0)	9** (1.1)
Nose	10	10	10	10	10	10
Exudate	0	0	0	0	1 (1.0)	1 (1.0)
Olfactory Epithelium, Atrophy	0	0	0	0	0	1 (2.0)
Olfactory Epithelium, Necrosis	0	0	0	1 (1.0)	0	0

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

800 mg/kg were greater than those in the vehicle controls, reaching statistical significance in the 800 mg/kg females. Minimal chronic active inflammation of the forestomach was seen in a single 50 mg/kg female.

Microscopically, hyperplasia was characterized by an increase in epithelial thickness greater than the normal three to four cells thick. Forestomach chronic active inflammation commonly accompanied the hyperplasia, and the forestomach degeneration was characterized by pallor and hydropic change of the hyperplastic epithelial cells in a diffuse or focally extensive pattern (Plates 1 and 2). Occasional pyknosis of epithelial nuclei in affected regions was also present.

In the nose, nasal exudate and olfactory epithelial atrophy or necrosis occurred occasionally in females receiving 400 or 800 mg/kg, and in males receiving 200 or 400 mg/kg, and the incidences of exudate and atrophy were significantly increased in 800 mg/kg males (Tables 4, A1, and A2). Olfactory epithelial atrophy was located in levels II and III, particularly in the dorsal and lateral areas of level III, and was characterized by the presence of shortened to flattened disorganized olfactory epithelium. Exudate in the lumen of the nasal passages consisted of mononuclear inflammatory cells and ghost forms of inflammatory cells in a coagulum of mucoid basophilic material.

MICE
2-WEEK STUDY

All 3,600 mg/kg mice were found dead or sacrificed moribund by day 9 (Table 5). One 1,200 mg/kg male and one 1,200 mg/kg female were found dead on days 12 and 16, respectively. The mean body weight gain of surviving 1,200 mg/kg males was significantly less than that of the vehicle controls, and surviving 1,200 mg/kg females lost weight during the study. Diarrhea, lethargy, thinness, and ruffled fur occurred in the 1,200 and 3,600 mg/kg groups.

Gross observations at necropsy included ulcerations of the stomach and adhesions involving the stomach and other abdominal organs in 1,200 and 3,600 mg/kg males and females. Microscopically, forestomach lesions in these groups included treatment-related ulceration in males and females and/or necrosis in females. Forestomach lesions were of sufficient severity to account for the morbidity and mortality of animals in these dose groups.

TABLE 5
Survival and Body Weights of Mice in the 2-Week Gavage Study of 2,4-Decadienal

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	23.0 ± 0.4	25.2 ± 0.5	2.2 ± 0.3	
45	5/5	22.2 ± 0.4	24.5 ± 0.4	2.3 ± 0.3	97
133	5/5	22.4 ± 0.5	24.7 ± 0.6	2.3 ± 0.3	98
400	5/5	22.5 ± 0.4	24.6 ± 0.7	2.1 ± 0.4	98
1,200	4/5[c]	22.6 ± 0.2	23.4 ± 0.4	0.7 ± 0.4*	93
3,600	0/5[d]	22.8 ± 0.6	—	—	—
Female					
0	5/5	18.8 ± 0.5	19.4 ± 0.4	0.6 ± 0.4	
45	5/5	19.3 ± 0.8	19.9 ± 0.8	0.6 ± 0.2	103
133	4/5	18.8 ± 0.3	20.0 ± 0.4	1.3 ± 0.1	103
400	5/5	18.8 ± 0.6	20.2 ± 0.7	1.4 ± 0.7	104
1,200	4/5[e]	18.9 ± 0.4	16.5 ± 0.3**	–2.3 ± 0.6**	85
3,600	0/5[f]	18.6 ± 0.5	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 17 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Day of death: 12
[d] Day of death: 3, 3, 3, 4, 7
[e] Day of death: 16
[f] Day of death: 3, 3, 3, 8, 9

Dose Selection Rationale: Due to high mortality in the 3,600 mg/kg groups and forestomach lesions in the 1,200 and 3,600 mg/kg groups, doses selected for the 3-month study in mice were 0, 50, 100, 200, 400, and 800 mg/kg.

3-Month Study

One 100 mg/kg and one 800 mg/kg female died due to dosing accidents during the second week of the study; all other mice survived to study termination (Table 6). Final mean body weights and body weight gains of 800 mg/kg males were significantly less than those of the vehicle controls (Table 6 and Figure 2). Salivation occurred in all males and females administered 400 or 800 mg/kg during weeks 7 through 10; females in the 200 mg/kg and greater groups were lethargic during week 12.

Table 6
Survival and Body Weights of Mice in the 3-Month Gavage Study of 2,4-Decadienal

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.2 ± 0.4	39.7 ± 1.0	16.5 ± 0.7	
50	10/10	23.5 ± 0.4	39.1 ± 0.9	15.5 ± 0.6	98
100	10/10	23.1 ± 0.3	40.1 ± 0.7	17.0 ± 0.5	101
200	10/10	23.1 ± 0.3	39.2 ± 1.0	16.1 ± 0.9	99
400	10/10	23.2 ± 0.3	38.8 ± 0.6	15.7 ± 0.7	98
800	10/10	23.3 ± 0.3	35.7 ± 0.7**	12.3 ± 0.6**	90
Female					
0	10/10	19.2 ± 0.3	32.3 ± 1.0	13.1 ± 0.9	
50	10/10	18.8 ± 0.2	30.8 ± 0.9	12.1 ± 0.8	96
100	9/10[c]	19.0 ± 0.3	33.0 ± 0.9	14.0 ± 0.6	102
200	10/10	18.8 ± 0.3	34.1 ± 0.8	15.3 ± 0.8	106
400	10/10	18.6 ± 0.2	31.1 ± 0.9	12.4 ± 0.8	96
800	9/10[d]	17.7 ± 0.5**	30.0 ± 1.3	12.5 ± 1.22	93

** Significantly different ($P \leq 0.01$) 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. Subsequent calculations are based on animals surviving to the end of the study.
[c] Day of death: 8
[d] Day of death: 10

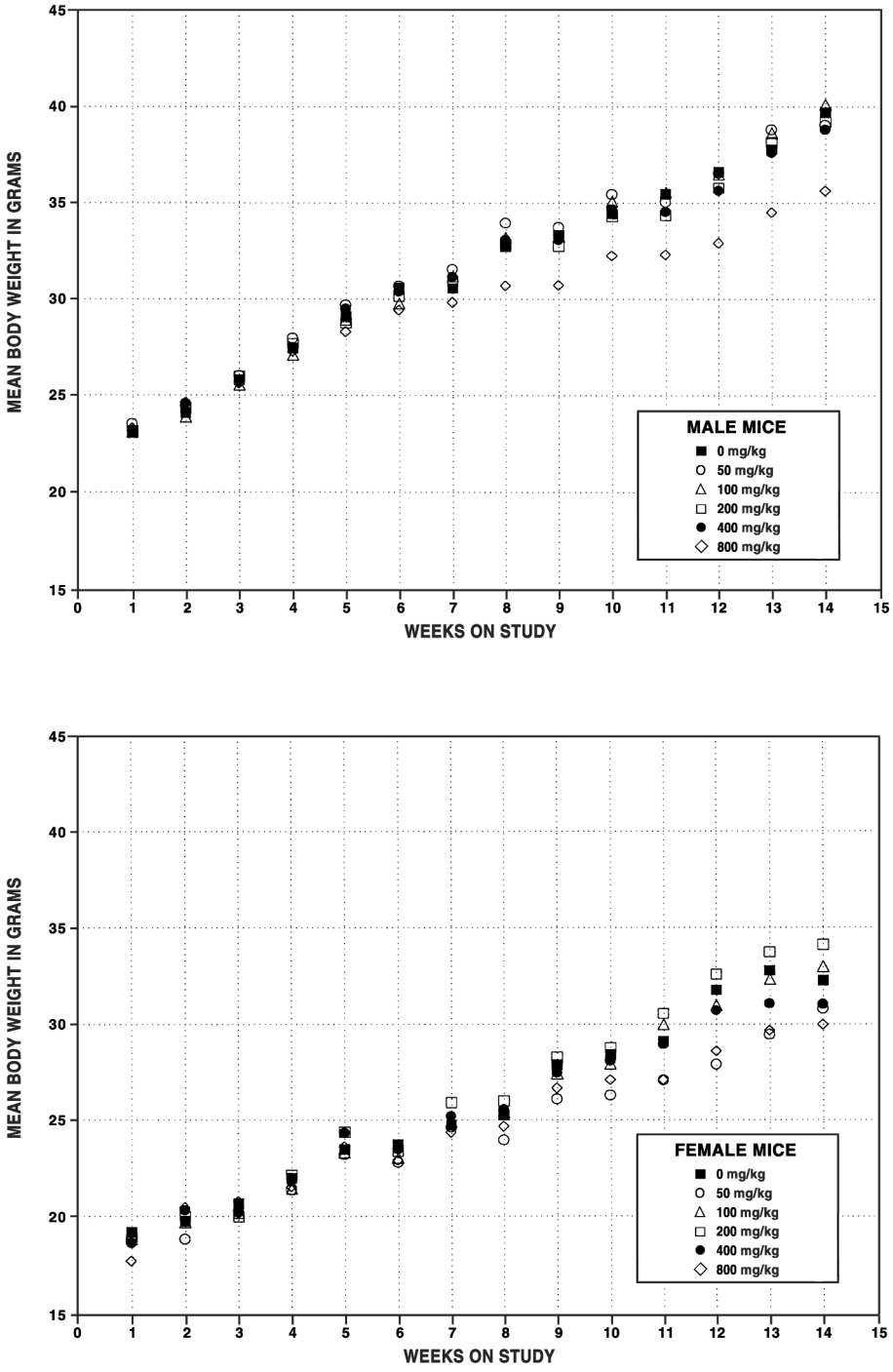


FIGURE 2
Body Weights of Male and Female Mice Administered 2,4-Decadienal by Gavage for 3 Months

The hematology data for mice in the 3-month toxicity study of 2,4-decadienal are listed in Table B2. Apparent, minimal (≤10%), treatment-related but not dose-related, decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts occurred in the higher-dosed male and/or female mice. This minimal change in the erythron was characterized as normocytic and normochromic and, while this may be a treatment effect, would be of questionable clinical significance.

Decreased organ weights in 800 mg/kg males compared to those in the vehicle controls were considered not to be treatment related but reflected decreased body weights in this group (Table C4). Females in the 800 mg/kg group had a significantly longer estrous cycle than did the vehicle controls (Table D4).

No treatment-related gross lesions were observed at necropsy. Microscopically, males receiving 200 mg/kg or greater and 800 mg/kg females had significantly increased incidences of epithelial hyperplasia of the forestomach (Tables 7, A3, and A4). Epithelial hyperplasia was also seen in a single 400 mg/kg female. Epithelial erosion or ulceration of the forestomach occurred in occasional males from the 200, 400, and 800 mg/kg dose groups. Increased incidences of olfactory epithelial necrosis occurred in males and females receiving 200 mg/kg or greater. Hydropic degeneration of olfactory epithelium was seen in a single female receiving 100 mg/kg. Olfactory epithelial necrosis occurred primarily in levels II and III and consisted of cellular swelling, cytoplasmic hyalinization, and nuclear pyknosis and karyorrhexis.

GENETIC TOXICOLOGY

2,4-Decadienal (0.1 to 1,000 µg/plate) was tested independently in two laboratories for mutagenicity in *Salmonella typhimurium*. No mutagenicity was detected in strains TA97, TA98, TA100, TA102, TA104, or TA1535, with or without induced rat or hamster liver S9 activation enzymes (Table E1). Acute bone marrow micronucleus tests in laboratory rodents administered 2,4-decadienal by intraperitoneal injection yielded mixed results. In male rats, a single injection of 100 to 600 mg/kg body weight produced significant increases in micronucleated polychromatic erythrocytes 24 hours after treatment at three of four dose levels tested, but no confirmatory trial was conducted (Table E2). The P value for the trend test in this study was not significant due to a downturn in the response at the highest dose tested that produced marked toxicity. In male mice, a standard three-injection (25 to 200 mg/kg per day) micronucleus test yielded negative results in bone marrow polychromatic erythrocytes 24 hours after the third injection. However, 48 hours after a single intraperitoneal injection of 600 mg/kg 2,4-decadienal, a small but statistically significant increase in micronucleated polychromatic erythrocytes was observed in bone marrow of male mice (Table E3). Analysis of peripheral blood polychromatic erythrocytes in these same mice showed a dose-related increase in micronucleated cells, but the increase was not of sufficient magnitude for a positive call. Because

peripheral blood is the more relevant site for micronucleus assessment in erythrocytes 48 hours after treatment, the results of the acute micronucleus assays in male mice were judged to be equivocal overall. No increase in the frequency of micronucleated normochromatic erythrocytes was seen in peripheral blood of male or female mice administered 2,4-decadienal (50 to 800 mg/kg per day) in the 3-month gavage study (Table E4).

TABLE 7
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Gavage Study of 2,4-Decadienal

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Male						
Stomach, Forestomach[a]	10	10	10	10	10	10
Epithelium, Hyperplasia[b]	0	0	0	5* (1.2)[c]	6** (1.0)	4* (1.5)
Epithelium, Ulcer	0	0	0	2 (1.0)	1 (1.0)	2 (1.5)
Epithelium, Erosion	0	0	0	0	0	1 (1.0)
Nose	10	10	10	10	10	10
Olfactory Epithelium, Necrosis	0	0	0	5* (2.8)	3 (2.3)	5* (2.8)
Female						
Stomach, Forestomach	10	10	10	10	10	10
Epithelium, Hyperplasia	0	0	0	0	1 (1.0)	4* (2.0)
Nose	10	10	10	10	10	10
Olfactory Epithelium, Necrosis	0	0	0	6** (2.5)	5* (1.4)	2 (3.0)
Olfactory Epithelium, Degeneration, Hydropic	0	0	1 (1.0)	0	0	0

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

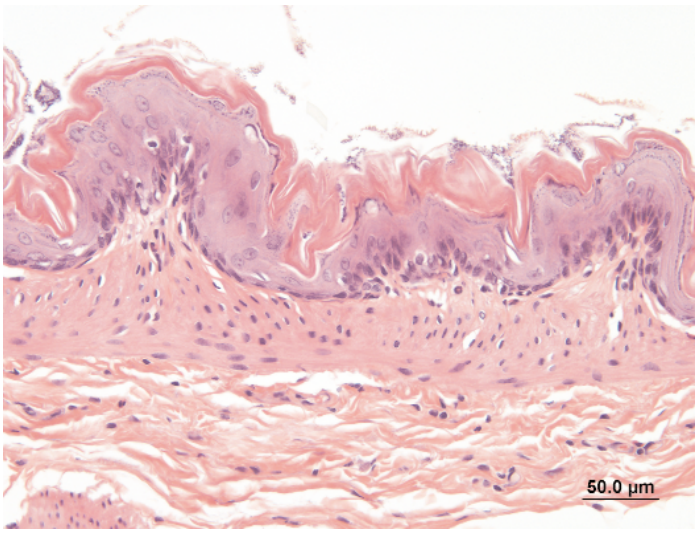


PLATE 1
Forestomach of a vehicle control female F344/N rat from the 3-month gavage study of 2,4-decadienal. The normal stratified squamous epithelium is two to three cells thick. H&E

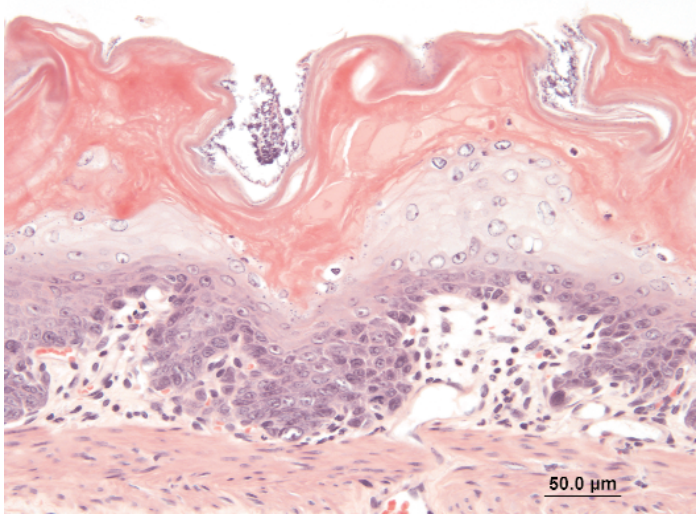


PLATE 2
Forestomach of a female F344/N rat administered 800 mg/kg 2,4-decadienal by gavage for 3 months. There is pallor and hydropic degeneration of hyperplastic epithelial cells, below which there is proliferation and downward projection of basal cells. The subepithelial connective tissue is inflamed. H&E

DISCUSSION

2,4-Decadienal and 2,4-hexadienal were nominated for study together because they are chemically and biologically similar. Both chemicals are auto-oxidation products of polyunsaturated fatty acids found in food, and both are used as food additive/flavoring agents. The potential for human exposure to these chemicals is high. The NTP conducted 2-week, 3-month, and 2-year studies of 2,4-hexadienal in rats and mice (Chan *et al*., 2003; NTP, 2003). The present report describes the 2-week and 3-month studies of 2,4-decadienal.

Treatment-related changes following gavage administration for up to 3 months were similar for both 2,4-hexadienal and 2,4-decadienal, and in both cases the forestomach and nose were identified as the target organs (Chan *et al*., 2003; NTP, 2003). In the 2-week studies of 2,4-hexadienal and 2,4-decadienal in rats and mice, forestomach lesions included necrosis and ulceration; epithelial hyperplasia was observed in rats and mice in the 2,4-hexadienal studies. In the 3-month studies of 2,4-hexadienal and 2,4-decadienal, forestomach epithelial hyperplasia and degeneration with or without chronic active inflammation occurred in addition to nasal olfactory epithelial atrophy or necrosis. In the current study, the no-observed-adverse-effect level was determined to be 100 mg/kg in rats and mice.

Carcinogenicity and mutagenicity data from the testing of dienals are limited. In the 2-year carcinogenicity studies, 2,4-hexadienal induced significantly increased incidences of forestomach neoplasms in male and female rats and mice (Chan *et al*., 2003; NTP, 2003). In the study of the mechanism of 2,4-hexadienal carcinogenesis, there were significant reductions in the GSH/GSSG ratio in male rats 4 hours after dosing, and DNA adducts (crotonaldehyde-dG 2) were significantly increased in the forestomach of male rats dosed with 90 mg/kg (NTP, 2003). Because 2,4-decadienal is less toxic than 2,4-hexadienal, 2-year studies were not conducted for this chemical.

2,4-Decadienal is one of the α,β-unsaturated aldehydes endogenously and exogenously formed from lipid peroxidation. α,β-Unsaturated aldehydes are direct-acting alkylating agents capable of covalent binding without prior metabolism to cellular nucleophilic groups (Witz, 1989). When reacting with 2-deoxyadenosine after epoxidation, 2,4-decadienal could generate DNA adducts (Loureiro *et al*., 2000; Carvalho *et al*., 2001). The etheno adducts produced could account for the cytotoxic properties. Incubation of 2,4-decadienal with human lung carcinoma (A-549) cells leading to the formation of 8-hydroxy-2′-deoxyguanosine has been demonstrated by Wu and Yen (2004). DNA-adducts may lead to miscoding during DNA replication, resulting, if not repaired, in mutations that can contribute to cancer development (Hageman *et al*., 1991).

In *in vitro* studies with human lung BEAS-2B cells, Chang *et al*. (2005) showed that 2,4-decadienal induced a dose-related increase in ROS production and a dose-related decrease in GSH/GSSG ratio. 2,4-Decadienal also induced the expression and release of pro-inflammatory cytokines TNF-α and IL-1β in the BEAS-2B cells. These authors also showed that a high dose of 2,4-decadienal was cytotoxic whereas a low dose stimulated cell proliferation. 2,4-Decadienal also induced ROS in human lung carcinoma (A-549) cells (Wu and Yen, 2004). The data confirmed the induction of oxidative stress by 2,4-decadienal at the target site.

Wu *et al*. (2001) reported in an abstract that 2,4-decadienal at 10 µg per plate was mutagenic in *Salmonella typhimurium* strains TA98 and TA100. Details of the study were not available. However, the present genotoxicity studies conducted in *S. typhimurium* strains TA97, TA98, TA100, TA102, TA104, and TA1535 at 0.1 to 1,000 µg per plate with or without S9 failed to demonstrate mutagenicity. Results of micronucleus assays were equivocal.

Although carcinogenicity studies of 2,4-decadienal have not been conducted, 2,4-decadienal could be expected to induce forestomach neoplasms in 2-year studies of rats and mice based on its structural similarity to 2,4-hexadienal, induction of similar nonneoplastic lesions in the forestomach after 3 months, and the mechanistic data cited above on DNA-adduct formation and oxidative stress. However, higher doses of 2,4-decadienal may be required to produce a neoplastic response. In the 3-month studies of 2,4-decadienal, the no-observed-adverse-effect level was 100 mg/kg in rats and mice. However, in the 2,4-hexadienal studies, equivalent doses (90 or 120 mg/kg) resulted in forestomach hyperplasia in the 3-month studies and forestomach neoplasms in the 2-year studies.

The forestomach is a relatively common target organ in NTP rodent bioassays, and the treatment-related changes seen with 2,4-decadienal are typical of those seen with exposure to irritants. Irritants can cause ulceration, inflammation, and hyperplasia, which is a known potential precursor lesion to neoplasia of the forestomach. Anatomically, the rodent forestomach, used for the temporary storage of food material prior to digestion, is lined by stratified squamous epithelium similar to that of the oral cavity and esophagus in humans. Similar histological changes could therefore occur in these sites in man; however, the duration of exposure to ingested material in humans is much shorter than in the forestomach of rodents.

Although olfactory epithelium atrophy and necrosis were observed in the 3-month studies of 2,4-hexadienal and 2,4-decadienal, no nasal lesions were observed in the 2-year study of 2,4-hexadienal. It is not known if nasal lesions would have occurred in a 2-year study of 2,4-decadienal. The nasal lesions may be due to irritation caused by these chemicals.

In summary, 2,4-decadienal administration caused decreased body weights and increased incidences of forestomach lesions in the 3-month studies in rats and mice. In addition, treatment-related lesions of the olfactory epithelium were observed in male rats and male and female mice. The no-observed-adverse-effect level was determined to be 100 mg/kg in rats and mice. 2,4-Decadienal was not mutagenic *in vitro* or *in vivo*.

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APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS AND MICE

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine small, duodenum	(10)					(10)
Erosion, focal						1 (10%)
Inflammation, chronic active						1 (10%)
Liver	(10)					(10)
Hepatodiaphragmatic nodule	1 (10%)					
Infiltration cellular, mixed cell	1 (10%)					1 (10%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Edema					2 (20%)	
Inflammation, chronic active					2 (20%)	2 (20%)
Epithelium, degeneration					1 (10%)	5 (50%)
Epithelium, hyperplasia					4 (40%)	6 (60%)
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	3 (30%)					
Endocrine System						
Adrenal cortex	(10)					(10)
Accessory adrenal cortical nodule	1 (10%)					
Pituitary gland	(10)					(10)
Pars distalis, cyst	1 (10%)					
Thyroid gland	(10)					(10)
Ultimobranchial cyst						1 (10%)
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Lymph node, mandibular	(10)					(10)
Hemorrhage	7 (70%)					4 (40%)
Lymph node, mesenteric	(10)					(10)
Hemorrhage	1 (10%)					
Thymus	(10)				(1)	(9)
Hemorrhage					1 (100%)	2 (22%)

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Gavage Study of 2,4-Decadienal

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Integumentary System						
Skin	(10)				(1)	(10)
Inflammation, chronic					1 (100%)	
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Hemorrhage	4 (40%)					3 (30%)
Infiltration cellular, histiocyte	1 (10%)					
Metaplasia, osseous						1 (10%)
Nose	(10)	(10)	(10)	(9)	(10)	(10)
Exudate					2 (20%)	6 (60%)
Inflammation, chronic	1 (10%)					1 (10%)
Olfactory epithelium, atrophy				1 (11%)	1 (10%)	4 (40%)
Olfactory epithelium, necrosis				1 (11%)		
Respiratory epithelium, hyperplasia						1 (10%)
Sinus, exudate				1 (11%)		
Turbinate, osteofibrosis				1 (11%)	1 (10%)	3 (30%)
Special Senses System						
None						
Urinary System						
None						

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

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)					(10)
Hepatodiaphragmatic nodule	4 (40%)					
Infiltration cellular, mixed cell	1 (10%)					
Mesentery	(1)			(1)		
Fat, necrosis	1 (100%)			1 (100%)		
Stomach, forestomach	(10)	(10)	(9)	(10)	(10)	(10)
Edema					1 (10%)	1 (10%)
Inflammation, chronic active		1 (10%)			2 (20%)	9 (90%)
Epithelium, degeneration					2 (20%)	10 (100%)
Epithelium, hyperplasia					6 (60%)	10 (100%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(10)					(10)
Accessory adrenal cortical nodule						1 (10%)
Pituitary gland	(10)					(10)
Pars distalis, cyst	1 (10%)					
Thyroid gland	(10)					(10)
Ultimobranchial cyst						1 (10%)
General Body System						
None						
Genital System						
Ovary	(10)			(1)	(1)	(10)
Cyst	1 (10%)			1 (100%)	1 (100%)	
Uterus	(10)			(3)		(10)
Hydrometra	4 (40%)			3 (100%)		2 (20%)
Hematopoietic System						
Lymph node, mandibular	(9)					(9)
Hemorrhage	2 (22%)					1 (11%)
Integumentary System						
None						

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 3-Month Gavage Study of 2,4-Decadienal

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Exudate					1 (10%)	1 (10%)
Olfactory epithelium, atrophy						1 (10%)
Olfactory epithelium, necrosis				1 (10%)		
Respiratory epithelium, inflammation			1 (10%)			
Respiratory epithelium, metaplasia, squamous					1 (10%)	
Special Senses System						
None						
Urinary System						
None						

[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 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(6)					(9)
Inflammation, chronic	1 (17%)					1 (11%)
Mesentery					(1)	
Necrosis, fatty					1 (100%)	
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Edema				2 (20%)		
Inflammation, acute				3 (30%)	1 (10%)	1 (10%)
Inflammation, chronic				1 (10%)	2 (20%)	1 (10%)
Epithelium, erosion						1 (10%)
Epithelium, hyperplasia				5 (50%)	6 (60%)	4 (40%)
Epithelium, ulcer				2 (20%)	1 (10%)	2 (20%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Lymph node, mesenteric	(10)			(1)		(10)
Infiltration cellular, plasma cell				1 (100%)		
Spleen	(10)		(1)	(1)		(10)
Hyperplasia, lymphoid				1 (100%)		
Pigmentation, hemosiderin			1 (100%)			
Thymus	(10)			(1)		(10)
Atrophy				1 (100%)		
Integumentary System						
None						
Musculoskeletal System						
Bone						

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 3-Month Gavage Study of 2,4-Decadienal

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Congestion	1 (10%)					
Infiltration cellular, histiocyte	1 (10%)					
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Exudate						1 (10%)
Olfactory epithelium, atrophy					1 (10%)	
Olfactory epithelium, metaplasia						1 (10%)
Olfactory epithelium, necrosis				5 (50%)	3 (30%)	5 (50%)
Respiratory epithelium, necrosis						3 (30%)
Special Senses System						
None						
Urinary System						
None						

[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 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental deaths			1			1
Survivors						
Terminal sacrifice	10	10	9	10	10	9
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(9)		(1)			(9)
Inflammation, acute						1 (11%)
Inflammation, chronic						1 (11%)
Perforation			1 (100%)			1 (11%)
Liver	(10)		(1)			(10)
Necrosis, focal						1 (10%)
Salivary glands	(10)		(1)			(10)
Inflammation, suppurative						1 (10%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Hyperkeratosis						1 (10%)
Inflammation, acute						1 (10%)
Inflammation, chronic						3 (30%)
Epithelium, hyperplasia					1 (10%)	4 (40%)
Stomach, glandular	(10)		(1)	(10)	(10)	(10)
Inflammation, acute						1 (10%)
Ulcer						1 (10%)
Cardiovascular System						
Heart	(10)		(1)			(10)
Atrium, inflammation, acute			1 (100%)			
Endocrine System						
Parathyroid gland	(9)					(7)
Cyst						1 (14%)
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Thymus	(10)		(1)			(10)
Necrosis, lymphoid			1 (100%)			
Integumentary System						
None						

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 3-Month Gavage Study of 2,4-Decadienal

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Musculoskeletal System						
Bone	(10)		(1)			(10)
Femur, fibrous osteodystrophy						1 (10%)
Nervous System						
None						
Respiratory System						
Lung	(10)		(1)			(10)
Inflammation, acute						1 (10%)
Mediastinum, necrosis			1 (100%)			
Perivascular, inflammation, chronic						1 (10%)
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Olfactory epithelium, atrophy					2 (20%)	1 (10%)
Olfactory epithelium, degeneration, hydropic			1 (10%)			
Olfactory epithelium, necrosis				6 (60%)	5 (50%)	2 (20%)
Sinus, inflammation, acute						1 (10%)
Sinus, respiratory epithelium, metaplasia, squamous						1 (10%)
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 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Male						
n						
Day 4	10	10	10	10	10	10
Day 19	10	10	9	10	10	10
Week 14	10	10	10	10	10	10
Hematology						
Automated hematocrit (%)						
Day 4	44.1 ± 0.5	45.1 ± 0.9	43.8 ± 0.6	45.0 ± 0.9	43.8 ± 0.8	46.0 ± 0.6
Day 19	45.1 ± 1.1	44.2 ± 0.5	44.8 ± 0.8	45.6 ± 0.5	44.5 ± 0.6	45.6 ± 0.4
Week 14	45.8 ± 0.3	44.3 ± 0.4	45.5 ± 0.4	45.1 ± 0.3	46.4 ± 0.5	48.6 ± 0.9*
Hemoglobin (g/dL)						
Day 4	14.7 ± 0.1	15.0 ± 0.3	14.6 ± 0.1	14.9 ± 0.2	14.5 ± 0.2	15.1 ± 0.2
Day 19	15.0 ± 0.3	14.8 ± 0.1	14.7 ± 0.3	15.3 ± 0.1	14.8 ± 0.2	15.1 ± 0.1
Week 14	15.3 ± 0.1	14.9 ± 0.1	14.9 ± 0.1	15.0 ± 0.1	15.3 ± 0.1	15.8 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	7.48 ± 0.07	7.65 ± 0.14	7.52 ± 0.06	7.64 ± 0.13	7.51 ± 0.09	8.01 ± 0.10**
Day 19	7.57 ± 0.16	7.57 ± 0.08	7.58 ± 0.13	7.76 ± 0.11	7.58 ± 0.11	7.73 ± 0.07
Week 14	9.02 ± 0.06	8.81 ± 0.08	9.01 ± 0.07	8.91 ± 0.05	9.14 ± 0.08	9.33 ± 0.17
Reticulocytes (10^5/µL)						
Day 4	4.10 ± 0.21	5.68 ± 0.21**	4.26 ± 0.16	5.65 ± 0.15**	5.46 ± 0.10**	4.94 ± 0.24
Day 19	3.62 ± 0.12	3.28 ± 0.15	3.19 ± 0.15	3.20 ± 0.12	3.10 ± 0.10**	2.67 ± 0.06**
Week 14	2.29 ± 0.04	2.38 ± 0.03	2.37 ± 0.07	2.46 ± 0.05*	2.51 ± 0.06**	2.66 ± 0.07**
Mean cell volume (fL)						
Day 4	59.0 ± 0.2	60.0 ± 0.4	58.2 ± 0.4	58.8 ± 0.3	58.3 ± 0.4	57.4 ± 0.4
Day 19	59.6 ± 0.3	58.4 ± 0.3	59.2 ± 0.6	58.8 ± 0.3	58.7 ± 0.4	59.1 ± 0.5
Week 14	50.8 ± 0.1	50.3 ± 0.2	50.6 ± 0.2	50.7 ± 0.2	50.8 ± 0.2	52.0 ± 0.1**
Mean cell hemoglobin (pg)						
Day 4	19.7 ± 0.2	19.6 ± 0.1	19.4 ± 0.2	19.6 ± 0.1	19.3 ± 0.1	18.9 ± 0.1**
Day 19	19.9 ± 0.1	19.5 ± 0.1	19.5 ± 0.1	19.7 ± 0.2	19.5 ± 0.1	19.5 ± 0.1
Week 14	17.0 ± 0.1	17.0 ± 0.0	16.6 ± 0.1*	16.9 ± 0.1	16.7 ± 0.1	16.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.4 ± 0.4	33.3 ± 0.3	33.4 ± 0.4	33.3 ± 0.3	33.0 ± 0.2	32.9 ± 0.3
Day 19	33.4 ± 0.3	33.5 ± 0.1	32.9 ± 0.2	33.5 ± 0.2	33.3 ± 0.1	33.1 ± 0.1
Week 14	33.4 ± 0.2	33.7 ± 0.2	32.8 ± 0.2*	33.3 ± 0.2	32.9 ± 0.2*	32.5 ± 0.2**
Platelets (10^3/µL)						
Day 4	884.5 ± 29.6	1,009.9 ± 27.3**	897.2 ± 37.4	931.7 ± 24.4	949.6 ± 18.1	975.5 ± 19.0*
Day 19	912.7 ± 45.0	916.4 ± 31.5	823.1 ± 27.9	807.4 ± 27.2	778.8 ± 24.5*	830.1 ± 27.3
Week 14	758.9 ± 28.9	759.4 ± 24.4	649.4 ± 18.0**	700.5 ± 12.3*	674.4 ± 13.2*	662.4 ± 8.1**
Leukocytes (10^3/µL)						
Day 4	8.88 ± 0.37	9.09 ± 0.22	8.76 ± 0.54	8.51 ± 0.53	8.79 ± 0.47	8.19 ± 0.29
Day 19	8.87 ± 0.39	9.35 ± 0.23	8.16 ± 0.50	9.08 ± 0.50	8.47 ± 0.32	7.68 ± 0.35*
Week 14	8.28 ± 0.33	8.50 ± 0.50	8.30 ± 0.43	7.71 ± 0.43	7.98 ± 0.53	5.94 ± 0.53**
Segmented neutrophils (10^3/µL)						
Day 4	0.91 ± 0.04	1.21 ± 0.04**	1.08 ± 0.08*	1.02 ± 0.08*	1.37 ± 0.08**	2.12 ± 0.18**
Day 19	0.90 ± 0.04	0.98 ± 0.07	0.75 ± 0.06	0.90 ± 0.03	1.03 ± 0.06	1.08 ± 0.08
Week 14	1.44 ± 0.04	1.35 ± 0.05	1.50 ± 0.12	1.52 ± 0.09	1.53 ± 0.09	1.85 ± 0.14*
Lymphocytes (10^3/µL)						
Day 4	7.72 ± 0.33	7.54 ± 0.22	7.40 ± 0.47	7.20 ± 0.47	7.10 ± 0.43	5.84 ± 0.22**
Day 19	7.58 ± 0.39	8.02 ± 0.19	7.10 ± 0.45	7.85 ± 0.49	7.10 ± 0.27	6.27 ± 0.32*
Week 14	6.44 ± 0.31	6.69 ± 0.44	6.38 ± 0.38	5.81 ± 0.38	6.05 ± 0.46	3.78 ± 0.38**
Monocytes (10^3/µL)						
Day 4	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01*	0.06 ± 0.00
Day 19	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Week 14	0.10 ± 0.01	0.13 ± 0.03	0.12 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.09 ± 0.01

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of 2,4-Decadienal

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Male (continued)						
n						
Day 4	10	10	10	10	10	10
Day 19	10	10	9	10	10	10
Week 14	10	10	10	10	10	10
Hematology (continued)						
Basophils (10^3/μL)						
Day 4	0.034 ± 0.003	0.062 ± 0.009*	0.040 ± 0.004	0.051 ± 0.008	0.055 ± 0.007	0.036 ± 0.004
Day 19	0.080 ± 0.009	0.088 ± 0.009	0.062 ± 0.008	0.063 ± 0.009	0.088 ± 0.021	0.060 ± 0.006
Week 14	0.056 ± 0.014	0.054 ± 0.010	0.051 ± 0.008	0.040 ± 0.010	0.049 ± 0.010	0.044 ± 0.009
Eosinophils (10^3/μL)						
Day 4	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.00	0.03 ± 0.00*
Day 19	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Week 14	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.00*	0.10 ± 0.02	0.06 ± 0.01**
Large unstained cells (10^3/μL)						
Day 4	0.128 ± 0.011	0.169 ± 0.014	0.137 ± 0.012	0.136 ± 0.012	0.149 ± 0.016	0.098 ± 0.008
Day 19	0.163 ± 0.013	0.152 ± 0.012	0.134 ± 0.013	0.140 ± 0.010	0.140 ± 0.023[b]	0.120 ± 0.012
Week 14	0.156 ± 0.010	0.177 ± 0.019	0.166 ± 0.014	0.151 ± 0.013	0.155 ± 0.015	0.118 ± 0.015
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 4	6.6 ± 0.4	8.4 ± 0.5*	7.9 ± 0.3*	8.9 ± 0.3**	9.3 ± 0.3**	12.8 ± 1.0**
Day 19	10.7 ± 0.4	10.2 ± 0.4	12.4 ± 0.7	10.6 ± 0.3	12.4 ± 0.7	10.7 ± 0.3
Week 14	12.3 ± 0.4	12.6 ± 0.5	13.4 ± 0.5	13.2 ± 0.4	12.6 ± 0.3	14.8 ± 0.8*
Creatinine (mg/dL)						
Day 4	0.64 ± 0.02	0.63 ± 0.03	0.59 ± 0.02	0.63 ± 0.03	0.64 ± 0.02	0.65 ± 0.03
Day 19	0.64 ± 0.02	0.64 ± 0.01	0.65 ± 0.01	0.66 ± 0.02	0.64 ± 0.02	0.61 ± 0.02
Week 14	0.85 ± 0.05	0.75 ± 0.02	0.78 ± 0.02	0.84 ± 0.04	0.77 ± 0.03	0.77 ± 0.03
Total protein (g/dL)						
Day 4	5.1 ± 0.0	5.7 ± 0.1**	5.2 ± 0.0*	5.7 ± 0.1**	5.7 ± 0.1**	5.8 ± 0.1**
Day 19	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.2 ± 0.1	6.0 ± 0.1
Week 14	6.4 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.3 ± 0.0	6.4 ± 0.1	6.7 ± 0.2
Albumin (g/dL)						
Day 4	3.6 ± 0.1	3.8 ± 0.1*	3.6 ± 0.0	3.9 ± 0.1*	4.0 ± 0.0**	3.9 ± 0.1**
Day 19	3.9 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.0 ± 0.1	4.2 ± 0.1	4.3 ± 0.1*
Week 14	4.5 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.7 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	61 ± 2	49 ± 2*	62 ± 2	48 ± 2*	46 ± 2**	73 ± 3
Day 19	53 ± 2	50 ± 1	53 ± 1	55 ± 2	53 ± 1	53 ± 3
Week 14	86 ± 8	76 ± 4	169 ± 19	92 ± 6	77 ± 8	57 ± 4**
Alkaline phosphatase (IU/L)						
Day 4	825 ± 30	806 ± 10	755 ± 20	760 ± 12	701 ± 14**	594 ± 17**
Day 19	661 ± 16	606 ± 12*	584 ± 13**	595 ± 13**	515 ± 12**	512 ± 8**
Week 14	289 ± 4	289 ± 6	274 ± 9	259 ± 6**	244 ± 10**	245 ± 6**
Creatine kinase (IU/L)						
Day 4	600 ± 149	508 ± 116	482 ± 55	519 ± 93	432 ± 89	393 ± 63
Day 19	716 ± 158	602 ± 170	431 ± 80	680 ± 156	566 ± 145	359 ± 76
Week 14	231 ± 34	162 ± 19	223 ± 31	222 ± 33	265 ± 46	221 ± 34
Sorbitol dehydrogenase (IU/L)						
Day 4	7 ± 1	8 ± 1	7 ± 0	6 ± 1	5 ± 1	5 ± 0
Day 19	11 ± 2	10 ± 1	6 ± 1*	9 ± 1	8 ± 1	5 ± 1**
Week 14	23 ± 3	20 ± 2	35 ± 4	25 ± 3	20 ± 5	12 ± 1**

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of 2,4-Decadienal

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Male (continued)						
n						
Day 4	10	10	10	10	10	10
Day 19	10	10	9	10	10	10
Week 14	10	10	10	10	10	10
Clinical Chemistry (continued)						
Bile acids (μmol/L)						
Day 4	22.5 ± 2.9	23.6 ± 3.1	25.6 ± 3.4	25.2 ± 3.6	20.4 ± 2.7	24.9 ± 2.6
Day 19	20.4 ± 2.1	21.6 ± 3.7	18.3 ± 2.4	24.5 ± 2.9	14.2 ± 1.4	15.8 ± 2.3
Week 14	23.0 ± 3.1	17.6 ± 2.3	21.5 ± 4.4	20.8 ± 3.6	18.3 ± 3.4	19.6 ± 2.1
Female						
n	10	10	10	10	10	10
Hematology						
Automated hematocrit (%)						
Day 4	44.4 ± 0.4	45.0 ± 0.6	44.3 ± 0.6	44.5 ± 0.5	44.9 ± 0.7	46.6 ± 0.8
Day 19	47.8 ± 0.7	46.4 ± 0.3	46.3 ± 0.5	46.1 ± 0.4	45.8 ± 0.6	46.1 ± 0.4
Week 14	44.4 ± 0.5	44.8 ± 0.4	43.9 ± 0.3	44.7 ± 0.5	44.4 ± 0.3	48.2 ± 0.5**
Hemoglobin (g/dL)						
Day 4	14.8 ± 0.1	15.0 ± 0.2	14.8 ± 0.1	14.7 ± 0.2	15.1 ± 0.2	15.6 ± 0.3
Day 19	16.2 ± 0.2	15.6 ± 0.1*	15.7 ± 0.2	15.6 ± 0.1*	15.4 ± 0.2**	15.5 ± 0.1**
Week 14	15.1 ± 0.1	15.1 ± 0.2	14.9 ± 0.1	14.9 ± 0.1	15.0 ± 0.2	16.0 ± 0.1**
Erythrocytes (10^6/μL)						
Day 4	7.64 ± 0.06	7.72 ± 0.09	7.71 ± 0.12	7.60 ± 0.09	7.85 ± 0.09	8.12 ± 0.16
Day 19	8.13 ± 0.09	7.94 ± 0.05	7.94 ± 0.07	7.85 ± 0.07	7.88 ± 0.08	7.86 ± 0.09
Week 14	8.21 ± 0.10	8.26 ± 0.08	8.11 ± 0.04	8.23 ± 0.07	8.19 ± 0.08	8.84 ± 0.08**
Reticulocytes (10^5/μL)						
Day 4	4.18 ± 0.19	4.55 ± 0.12	4.07 ± 0.17	4.82 ± 0.13*	4.08 ± 0.16	4.13 ± 0.20
Day 19	2.28 ± 0.10	2.15 ± 0.08	2.23 ± 0.06	2.40 ± 0.09	2.26 ± 0.09	2.38 ± 0.09
Week 14	2.12 ± 0.07	2.22 ± 0.09	2.05 ± 0.05	2.23 ± 0.07	2.09 ± 0.07	1.94 ± 0.09
Mean cell volume (fL)						
Day 4	58.2 ± 0.3	58.3 ± 0.1	57.6 ± 0.2	58.5 ± 0.3	57.2 ± 0.3*	57.5 ± 0.4
Day 19	58.8 ± 0.4	58.4 ± 0.3	58.3 ± 0.3	58.8 ± 0.4	58.2 ± 0.4	58.6 ± 0.3
Week 14	54.1 ± 0.1	54.2 ± 0.2	54.1 ± 0.2	54.4 ± 0.3	54.2 ± 0.2	54.6 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.4 ± 0.1	19.4 ± 0.1	19.2 ± 0.1	19.4 ± 0.1	19.2 ± 0.1	19.2 ± 0.1
Day 19	19.9 ± 0.1	19.7 ± 0.0	19.8 ± 0.1	19.9 ± 0.1	19.6 ± 0.1*	19.7 ± 0.1
Week 14	18.4 ± 0.1	18.3 ± 0.1	18.4 ± 0.1	18.2 ± 0.1	18.3 ± 0.1	18.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.3 ± 0.2	33.2 ± 0.2	33.4 ± 0.2	33.1 ± 0.2	33.6 ± 0.1	33.4 ± 0.2
Day 19	33.8 ± 0.2	33.7 ± 0.1	34.0 ± 0.1	33.8 ± 0.2	33.7 ± 0.2	33.6 ± 0.1
Week 14	34.0 ± 0.2	33.7 ± 0.2	34.0 ± 0.2	33.4 ± 0.2	33.7 ± 0.2	33.2 ± 0.2**
Platelets (10^3/μL)						
Day 4	880.1 ± 27.6	919.3 ± 18.6	900.5 ± 17.4	1,011.3 ± 24.5**	921.7 ± 17.3 .	962.5 ± 29.0
Day 19	868.5 ± 18.4	870.5 ± 24.1	903.2 ± 16.8	916.2 ± 26.6	869.5 ± 29.3	892.7 ± 17.5
Week 14	764.5 ± 15.8	747.3 ± 14.7[b]	718.5 ± 7.0	764.3 ± 12.0	745.2 ± 12.0	693.9 ± 11.4**
Leukocytes (10^3/μL)						
Day 4	10.81 ± 0.32	10.98 ± 0.34	11.91 ± 0.32	10.59 ± 0.23	9.78 ± 0.28	7.23 ± 0.35**
Day 19	10.14 ± 0.20	9.04 ± 0.37*	9.55 ± 0.32	8.65 ± 0.34**	8.54 ± 0.46**	8.11 ± 0.46**
Week 14	7.16 ± 0.28	8.08 ± 0.39	8.11 ± 0.28	7.99 ± 0.36	6.94 ± 0.43	5.64 ± 0.41

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of 2,4-Decadienal

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Female (continued)						
n	10	10	10	10	10	10
Hematology (continued)						
Segmented neutrophils (10^3/µL)						
Day 4	1.25 ± 0.12	1.16 ± 0.07	1.27 ± 0.10	0.90 ± 0.06*	1.00 ± 0.06	1.75 ± 0.19
Day 19	1.21 ± 0.05	1.01 ± 0.09	0.95 ± 0.06*	0.95 ± 0.05*	1.07 ± 0.06	1.31 ± 0.12
Week 14	1.32 ± 0.04	1.34 ± 0.09	1.52 ± 0.10	1.39 ± 0.09	1.40 ± 0.11	1.45 ± 0.11
Lymphocytes (10^3/µL)						
Day 4	9.09 ± 0.31	9.41 ± 0.31	10.14 ± 0.25	9.22 ± 0.20	8.40 ± 0.27	5.17 ± 0.29**
Day 19	8.56 ± 0.22	7.69 ± 0.33*	8.20 ± 0.29	7.36 ± 0.30**	7.10 ± 0.41**	6.43 ± 0.40**
Week 14	5.40 ± 0.22	6.24 ± 0.28	6.16 ± 0.21	6.12 ± 0.27	5.16 ± 0.38	3.90 ± 0.32
Monocytes (10^3/µL)						
Day 4	0.07 ± 0.00	0.07 ± 0.00	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.00	0.07 ± 0.01
Day 19	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Week 14	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.00	0.11 ± 0.01	0.09 ± 0.01	0.10 ± 0.01
Basophils (10^3/µL)						
Day 4	0.110 ± 0.017	0.096 ± 0.009	0.113 ± 0.011	0.121 ± 0.014	0.086 ± 0.009	0.049 ± 0.005**
Day 19	0.059 ± 0.006	0.065 ± 0.007	0.096 ± 0.011	0.059 ± 0.007	0.067 ± 0.009	0.079 ± 0.011
Week 14	0.078 ± 0.024	0.081 ± 0.018	0.060 ± 0.007	0.079 ± 0.014	0.051 ± 0.010	0.032 ± 0.007
Eosinophils (10^3/µL)						
Day 4	0.06 ± 0.00	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.04 ± 0.00
Day 19	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.00	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Week 14	0.09 ± 0.00	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01*	0.04 ± 0.01**
Large unstained cells (10^3/µL)						
Day 4	0.235 ± 0.031	0.195 ± 0.012	0.247 ± 0.018	0.220 ± 0.017	0.173 ± 0.014	0.162 ± 0.019*
Day 19	0.132 ± 0.012	0.123 ± 0.013	0.162 ± 0.012	0.140 ± 0.013	0.134 ± 0.010	0.136 ± 0.013
Week 14	0.201 ± 0.042	0.227 ± 0.033	0.177 ± 0.017	0.201 ± 0.026	0.172 ± 0.022	0.122 ± 0.015
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 4	7.2 ± 0.3	8.0 ± 0.4	8.7 ± 0.3**	8.8 ± 0.5**	9.0 ± 0.5**	11.7 ± 0.8**
Day 19	12.7 ± 0.5	12.7 ± 0.4	13.3 ± 0.5	12.5 ± 0.5	11.4 ± 0.5	13.3 ± 0.4
Week 14	10.6 ± 0.2	11.8 ± 0.3	11.2 ± 0.4	11.4 ± 0.4	10.6 ± 0.4	9.9 ± 0.4
Creatinine (mg/dL)						
Day 4	0.63 ± 0.01	0.65 ± 0.02	0.65 ± 0.01	0.67 ± 0.01	0.66 ± 0.01	0.63 ± 0.02
Day 19	0.58 ± 0.02	0.57 ± 0.02	0.56 ± 0.01	0.56 ± 0.03	0.60 ± 0.03	0.55 ± 0.02
Week 14	0.68 ± 0.02	0.69 ± 0.02	0.67 ± 0.02	0.67 ± 0.02	0.67 ± 0.01	0.65 ± 0.02
Total protein (g/dL)						
Day 4	5.5 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	6.1 ± 0.2**
Day 19	6.1 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.1 ± 0.1
Week 14	6.5 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.2 ± 0.1*	6.2 ± 0.1*
Albumin (g/dL)						
Day 4	3.7 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	4.0 ± 0.1**	4.2 ± 0.1**
Day 19	4.2 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.3 ± 0.1
Week 14	4.8 ± 0.1	4.7 ± 0.1	4.8 ± 0.1	4.7 ± 0.1	4.5 ± 0.1	4.6 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	47 ± 3	43 ± 2	49 ± 3	45 ± 2	44 ± 2	85 ± 7**
Day 19	46 ± 2	45 ± 2	46 ± 2	43 ± 2	44 ± 2	47 ± 3
Week 14	67 ± 7	57 ± 4	65 ± 5	50 ± 2	53 ± 2	56 ± 2
Alkaline phosphatase (IU/L)						
Day 4	710 ± 27	690 ± 31	691 ± 11	696 ± 20	578 ± 22**	566 ± 27**
Day 19	503 ± 16	488 ± 15	490 ± 7	507 ± 10	453 ± 15*	446 ± 11**
Week 14	262 ± 5	266 ± 5	257 ± 8	242 ± 9	244 ± 10	262 ± 5

TABLE B1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of 2,4-Decadienal

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Female (continued)						
n	10	10	10	10	10	10
Clinical Chemistry (continued)						
Creatine kinase (IU/L)						
Day 4	306 ± 24	312 ± 30	340 ± 26	286 ± 35	283 ± 30	302 ± 24
Day 19	471 ± 117	431 ± 44	440 ± 88	318 ± 55	294 ± 53[b]	319 ± 42
Week 14	182 ± 25	212 ± 57	259 ± 62	199 ± 27	191 ± 25	240 ± 45
Sorbitol dehydrogenase (IU/L)						
Day 4	5 ± 1	5 ± 1	5 ± 1	5 ± 1	4 ± 1	4 ± 1
Day 19	8 ± 1	8 ± 1	8 ± 1	8 ± 1	7 ± 1	7 ± 1
Week 14	10 ± 1	9 ± 1	10 ± 1	7 ± 1	7 ± 1	6 ± 1**
Bile acids (μmol/L)						
Day 4	21.4 ± 2.8	20.3 ± 2.6	20.1 ± 1.5	22.2 ± 2.2	25.6 ± 1.8	18.7 ± 2.2
Day 19	14.3 ± 2.3	15.6 ± 2.0	18.4 ± 2.5	26.6 ± 3.4*	28.7 ± 4.9*	18.2 ± 2.6
Week 14	11.0 ± 2.2	15.6 ± 2.0	19.3 ± 2.5*	13.2 ± 1.3	14.4 ± 1.7	13.2 ± 1.4

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

TABLE B2
Hematology Data for Mice in the 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Male						
n	10	10	10	10	10	10
Automated hematocrit (%)	49.4 ± 1.1	50.0 ± 0.2	49.2 ± 0.8	44.4 ± 1.3*	46.6 ± 0.8*	47.5 ± 0.7*
Hemoglobin (g/dL)	16.7 ± 0.3	16.8 ± 0.1	16.4 ± 0.3	15.0 ± 0.4**	15.7 ± 0.2**	15.8 ± 0.3**
Erythrocytes (10^6/µL)	11.03 ± 0.25	11.12 ± 0.08	10.99 ± 0.21	10.02 ± 0.27*	10.48 ± 0.19*	10.65 ± 0.17*
Reticulocytes (10^5/µL)	4.18 ± 0.19[b]	4.08 ± 0.13	3.95 ± 0.15	3.53 ± 0.15*[b]	3.67 ± 0.18*	3.66 ± 0.13*[b]
Mean cell volume (fL)	44.8 ± 0.1	45.0 ± 0.2	44.8 ± 0.3	44.3 ± 0.4	44.5 ± 0.2	44.6 ± 0.2
Mean cell hemoglobin (pg)	15.1 ± 0.1	15.1 ± 0.1	15.0 ± 0.1	15.0 ± 0.1	15.0 ± 0.1	14.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.7 ± 0.2	33.5 ± 0.1	33.4 ± 0.2	33.9 ± 0.2	33.7 ± 0.2	33.3 ± 0.2
Platelets (10^3/µL)	1,198.3 ± 44.3	1,316.6 ± 30.4	1,185.1 ± 48.9	1,177.0 ± 31.1	1,229.4 ± 35.5	1,254.9 ± 38.7
Leukocytes (10^3/µL)	3.48 ± 0.28	3.84 ± 0.27	3.46 ± 0.32	4.21 ± 0.59	3.92 ± 0.31	4.50 ± 0.55
Segmented neutrophils (10^3/µL)	0.56 ± 0.05	0.65 ± 0.06	0.53 ± 0.05	1.35 ± 0.52	0.59 ± 0.04	0.71 ± 0.09
Lymphocytes (10^3/µL)	2.72 ± 0.24	2.97 ± 0.21	2.68 ± 0.24	2.66 ± 0.22	3.09 ± 0.27	3.55 ± 0.45
Monocytes (10^3/µL)	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Basophils (10^3/µL)	0.008 ± 0.002	0.006 ± 0.003	0.010 ± 0.002	0.009 ± 0.003	0.013 ± 0.002	0.011 ± 0.003
Eosinophils (10^3/µL)	0.13 ± 0.01	0.14 ± 0.02	0.16 ± 0.02	0.12 ± 0.03	0.17 ± 0.03	0.16 ± 0.04
Large unstained cells (10^3/µL)	0.014 ± 0.004[c]	0.016 ± 0.003[d]	0.017 ± 0.003[b]	0.016 ± 0.003[b]	0.014 ± 0.004	0.014 ± 0.003[d]
Female						
n	10	10	9	10	10	10
Automated hematocrit (%)	46.7 ± 0.9	45.7 ± 0.8	45.8 ± 0.7	45.0 ± 0.3	45.4 ± 0.4	44.6 ± 0.3
Hemoglobin (g/dL)	15.6 ± 0.2	15.3 ± 0.2	15.4 ± 0.2	15.1 ± 0.1*	15.1 ± 0.1*	14.7 ± 0.1**
Erythrocytes (10^6/µL)	10.27 ± 0.18	9.98 ± 0.18	9.94 ± 0.13	9.88 ± 0.08	10.02 ± 0.09	9.80 ± 0.08
Reticulocytes (10^5/µL)	3.32 ± 0.18	3.72 ± 0.24	3.70 ± 0.27	3.12 ± 0.19[b]	3.12 ± 0.13	3.09 ± 0.18[d]
Mean cell volume (fL)	45.4 ± 0.2	45.8 ± 0.2	46.1 ± 0.2	45.5 ± 0.3	45.3 ± 0.2	45.6 ± 0.1
Mean cell hemoglobin (pg)	15.2 ± 0.1	15.3 ± 0.1	15.5 ± 0.1	15.3 ± 0.1	15.1 ± 0.5	15.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.5 ± 0.2	33.5 ± 0.2	33.6 ± 0.2	33.6 ± 0.1	33.2 ± 0.2	33.0 ± 0.1
Platelets (10^3/µL)	1,008.6 ± 41.1	1,130.5 ± 45.7	1,038.3 ± 53.6	1,036.8 ± 40.5	946.5 ± 56.4	907.4 ± 18.4
Leukocytes (10^3/µL)	2.91 ± 0.16	3.58 ± 0.25	3.08 ± 0.16	3.38 ± 0.41	3.26 ± 0.19	3.42 ± 0.16
Segmented neutrophils (10^3/µL)	0.45 ± 0.06	0.56 ± 0.05	0.51 ± 0.08	0.70 ± 0.16	0.42 ± 0.05	0.56 ± 0.07
Lymphocytes (10^3/µL)	2.31 ± 0.13	2.87 ± 0.21	2.46 ± 0.12	2.52 ± 0.34	2.69 ± 0.17	2.70 ± 0.19
Basophils (10^3/µL)	0.003 ± 0.002	0.008 ± 0.001	0.007 ± 0.002	0.007 ± 0.003	0.005 ± 0.003	0.008 ± 0.002
Monocytes (10^3/µL)	0.06 ± 0.02	0.04 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Eosinophils (10^3/µL)	0.09 ± 0.02	0.09 ± 0.02	0.06 ± 0.01	0.09 ± 0.02	0.10 ± 0.02	0.09 ± 0.02
Large unstained cells (10^3/µL)	0.017 ± 0.004[c]	0.023 ± 0.002[d]	0.012 ± 0.002[e]	0.020 ± 0.004[f]	0.017 ± 0.007[g]	0.017 ± 0.003[f]

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=7
[d] n=8
[e] n=5
[f] n=6
[g] n=3

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 2-Week Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	45 mg/kg	133 mg/kg	400 mg/kg	1,200 mg/kg	3,600 mg/kg
Male						
n	5	5	5	5	5	0[b]
Necropsy body wt	179 ± 5	184 ± 7	176 ± 4	168 ± 6	140 ± 10**	
R. Kidney						
Absolute	0.736 ± 0.021	0.774 ± 0.050	0.736 ± 0.014	0.678 ± 0.015	0.631 ± 0.029*	
Relative	4.122 ± 0.015	4.192 ± 0.127	4.185 ± 0.091	4.044 ± 0.085	4.555 ± 0.134*	
Liver						
Absolute	9.067 ± 0.206	9.879 ± 0.510	9.106 ± 0.230	8.788 ± 0.456	7.552 ± 0.559	
Relative	50.824 ± 0.631	53.596 ± 1.320	51.733 ± 0.734	52.191 ± 1.485	54.073 ± 1.089	
Female						
n	5	5	4	5	5	0[b]
Necropsy body wt	130 ± 4	131 ± 6	131 ± 6	135 ± 4	110 ± 5*	
R. Kidney						
Absolute	0.558 ± 0.018	0.555 ± 0.023	0.571 ± 0.023	0.562 ± 0.016	0.509 ± 0.015	
Relative	4.305 ± 0.029	4.238 ± 0.087	4.357 ± 0.027	4.177 ± 0.037	4.650 ± 0.136*	
Liver						
Absolute	5.804 ± 0.115	5.986 ± 0.238	5.973 ± 0.141	6.120 ± 0.177	5.465 ± 0.333	
Relative	44.863 ± 0.527	45.727 ± 0.730	45.663 ± 1.069	45.518 ± 1.034	49.668 ± 1.547*	

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] No data were available for the 3,600 mg/kg groups due to 100% mortality.

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	333 ± 6	341 ± 9	330 ± 5	323 ± 7	307 ± 5**	280 ± 7**
Heart						
Absolute	0.875 ± 0.019	0.910 ± 0.031	0.857 ± 0.014	0.873 ± 0.025	0.826 ± 0.027	0.753 ± 0.014**
Relative	2.626 ± 0.026	2.668 ± 0.038	2.601 ± 0.029	2.698 ± 0.046	2.689 ± 0.084	2.692 ± 0.044
R. Kidney						
Absolute	0.956 ± 0.027	1.004 ± 0.035	0.941 ± 0.039	0.995 ± 0.027	0.912 ± 0.014	0.824 ± 0.027**
Relative	2.868 ± 0.058	2.947 ± 0.066	2.847 ± 0.088	3.077 ± 0.058	2.970 ± 0.041	2.935 ± 0.041
Liver						
Absolute	10.252 ± 0.198	11.039 ± 0.389	10.569 ± 0.354	10.449 ± 0.208	9.465 ± 0.178	7.794 ± 0.242**
Relative	30.778 ± 0.364	32.362 ± 0.511	32.005 ± 0.703	32.330 ± 0.383	30.825 ± 0.580	27.774 ± 0.341**
Lung						
Absolute	1.429 ± 0.060	1.286 ± 0.040*	1.307 ± 0.060	1.260 ± 0.036*	1.219 ± 0.031**	1.079 ± 0.042**
Relative	4.277 ± 0.124	3.785 ± 0.114*	3.952 ± 0.127	3.893 ± 0.063	3.972 ± 0.110	3.848 ± 0.106*
Spleen						
Absolute	0.593 ± 0.012	0.615 ± 0.016	0.591 ± 0.012	0.597 ± 0.017	0.530 ± 0.012**	0.439 ± 0.009**
Relative	1.781 ± 0.026	1.807 ± 0.031	1.794 ± 0.036	1.846 ± 0.039	1.725 ± 0.030	1.569 ± 0.026**
R. Testis						
Absolute	1.419 ± 0.026	1.476 ± 0.024	1.396 ± 0.018	1.429 ± 0.023	1.415 ± 0.016	1.309 ± 0.031**
Relative	4.265 ± 0.077	4.348 ± 0.084	4.240 ± 0.055	4.428 ± 0.080	4.607 ± 0.046**	4.676 ± 0.062**
Thymus						
Absolute	0.261 ± 0.012	0.265 ± 0.008	0.245 ± 0.014	0.261 ± 0.008	0.244 ± 0.008	0.185 ± 0.007**
Relative	0.783 ± 0.028	0.783 ± 0.032	0.743 ± 0.038	0.808 ± 0.018	0.794 ± 0.026	0.661 ± 0.026*
Female						
Necropsy body wt	199 ± 3	197 ± 4	195 ± 3	189 ± 2	195 ± 3	189 ± 3
Heart						
Absolute	0.594 ± 0.014	0.610 ± 0.010	0.588 ± 0.010	0.584 ± 0.003	0.587 ± 0.010	0.564 ± 0.014
Relative	2.990 ± 0.059	3.106 ± 0.054	3.018 ± 0.028	3.089 ± 0.029	3.014 ± 0.049	2.982 ± 0.070
R. Kidney						
Absolute	0.642 ± 0.011	0.678 ± 0.011	0.633 ± 0.009	0.641 ± 0.012	0.640 ± 0.014	0.610 ± 0.016
Relative	3.232 ± 0.048	3.450 ± 0.045*	3.251 ± 0.037	3.390 ± 0.067	3.282 ± 0.048	3.221 ± 0.057
Liver						
Absolute	5.654 ± 0.096	5.726 ± 0.184	5.737 ± 0.129	5.577 ± 0.111	5.385 ± 0.118	5.307 ± 0.165
Relative	28.454 ± 0.302	29.073 ± 0.596	29.479 ± 0.694	29.482 ± 0.534	27.614 ± 0.381	28.005 ± 0.605
Lung						
Absolute	0.900 ± 0.014	0.964 ± 0.032	0.935 ± 0.027	0.922 ± 0.020	0.926 ± 0.027	0.856 ± 0.017
Relative	4.531 ± 0.052	4.905 ± 0.153	4.800 ± 0.122	4.876 ± 0.107	4.743 ± 0.084	4.524 ± 0.072
Spleen						
Absolute	0.410 ± 0.008	0.432 ± 0.011	0.414 ± 0.007	0.423 ± 0.014	0.391 ± 0.019	0.328 ± 0.012**
Relative	2.066 ± 0.045	2.195 ± 0.037	2.125 ± 0.023	2.237 ± 0.073	1.999 ± 0.081	1.732 ± 0.059**
Thymus						
Absolute	0.205 ± 0.009	0.206 ± 0.010	0.210 ± 0.006	0.202 ± 0.007	0.179 ± 0.012*	0.153 ± 0.005**
Relative	1.032 ± 0.042	1.044 ± 0.038	1.079 ± 0.033	1.068 ± 0.031	0.915 ± 0.054*	0.809 ± 0.027**

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

TABLE C3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	45 mg/kg	133 mg/kg	400 mg/kg	1,200 mg/kg	3,600 mg/kg
n	5	5	5	5	4	0[b]
Male						
Necropsy body wt	25.2 ± 0.5	24.5 ± 0.4	24.7 ± 0.6	24.6 ± 0.7	23.4 ± 0.4	
R. Kidney						
Absolute	0.246 ± 0.008	0.217 ± 0.009	0.230 ± 0.008	0.227 ± 0.010	0.225 ± 0.007	
Relative	9.770 ± 0.173	8.877 ± 0.361	9.292 ± 0.195	9.238 ± 0.325	9.624 ± 0.225	
Liver						
Absolute	1.480 ± 0.033	1.412 ± 0.039	1.432 ± 0.068	1.427 ± 0.077	1.451 ± 0.080	
Relative	58.786 ± 0.816	57.633 ± 1.528	57.830 ± 1.832	57.894 ± 1.937	62.030 ± 2.589	
Female						
Necropsy body wt	19.4 ± 0.4	19.9 ± 0.8	20.0 ± 0.4	20.2 ± 0.7	16.5 ± 0.3**	
R. Kidney						
Absolute	0.141 ± 0.003	0.144 ± 0.010	0.144 ± 0.002	0.142 ± 0.003	0.130 ± 0.003	
Relative	7.261 ± 0.105	7.201 ± 0.288	7.207 ± 0.193	7.039 ± 0.189	7.921 ± 0.303	
Liver						
Absolute	0.961 ± 0.019	0.967 ± 0.056	0.984 ± 0.33	0.997 ± 0.047	0.857 ± 0.030	
Relative	49.544 ± 0.449	48.444 ± 1.161	49.076 ± 1.057	49.413 ± 1.439	52.114 ± 2.500	

** Significantly different ($P \leq 0.01$) from the vehicle control group by Dunnett's test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] No data were available for the 3,600 mg/kg groups due to 100% mortality.

TABLE C4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	39.4 ± 0.9	39.2 ± 0.8	39.1 ± 0.6	38.1 ± 1.0	38.5 ± 0.7	35.4 ± 0.7**
Heart						
Absolute	0.159 ± 0.005	0.153 ± 0.003	0.147 ± 0.004	0.162 ± 0.004	0.142 ± 0.004**	0.140 ± 0.004**
Relative	4.044 ± 0.123	3.914 ± 0.084	3.771 ± 0.109	4.255 ± 0.082	3.694 ± 0.093	3.951 ± 0.095
R. Kidney						
Absolute	0.295 ± 0.011	0.308 ± 0.008	0.299 ± 0.005	0.307 ± 0.010	0.295 ± 0.007	0.280 ± 0.007
Relative	7.519 ± 0.311	7.866 ± 0.181	7.664 ± 0.122	8.062 ± 0.190	7.672 ± 0.153	7.914 ± 0.191
Liver						
Absolute	1.371 ± 0.052	1.325 ± 0.054	1.336 ± 0.043	1.333 ± 0.024	1.315 ± 0.030	1.171 ± 0.035**
Relative	34.847 ± 1.200	33.898 ± 1.400	34.161 ± 0.754	35.160 ± 1.075	34.197 ± 0.670	33.029 ± 0.594
Lung						
Absolute	0.209 ± 0.017	0.177 ± 0.010	0.160 ± 0.003**	0.200 ± 0.009	0.174 ± 0.008	0.167 ± 0.005*[b]
Relative	5.270 ± 0.311	4.507 ± 0.206	4.102 ± 0.075**	5.285 ± 0.293	4.514 ± 0.169	4.745 ± 0.198[b]
Spleen						
Absolute	0.076 ± 0.004	0.071 ± 0.002	0.071 ± 0.003	0.098 ± 0.014	0.069 ± 0.004	0.062 ± 0.002
Relative	1.930 ± 0.089	1.816 ± 0.049	1.823 ± 0.099	2.664 ± 0.498	1.784 ± 0.067	1.760 ± 0.086
R. Testis						
Absolute	0.116 ± 0.003	0.115 ± 0.002	0.104 ± 0.003*	0.111 ± 0.003	0.113 ± 0.003	0.112 ± 0.004
Relative	2.963 ± 0.066	2.945 ± 0.107	2.675 ± 0.086	2.934 ± 0.084	2.943 ± 0.082	3.176 ± 0.133
Thymus						
Absolute	0.030 ± 0.002	0.033 ± 0.002	0.032 ± 0.002	0.030 ± 0.002	0.027 ± 0.002	0.031 ± 0.001
Relative	0.770 ± 0.047	0.828 ± 0.056	0.807 ± 0.048	0.782 ± 0.055	0.701 ± 0.043	0.884 ± 0.035

TABLE C4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of 2,4-Decadienal

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Female						
n	10	10	9	10	10	9
Necropsy body wt	32.5 ± 0.9	31.1 ± 1.0	33.0 ± 0.8	33.9 ± 1.0	31.1 ± 0.9	29.8 ± 1.3
Heart						
Absolute	0.123 ± 0.004	0.131 ± 0.005	0.137 ± 0.006	0.118 ± 0.003	0.127 ± 0.004	0.123 ± 0.006
Relative	3.802 ± 0.121	4.268 ± 0.233	4.149 ± 0.153	3.506 ± 0.118	4.109 ± 0.150	4.206 ± 0.266
R. Kidney						
Absolute	0.175 ± 0.003	0.190 ± 0.007	0.193 ± 0.009	0.172 ± 0.005	0.183 ± 0.005	0.188 ± 0.008
Relative	5.430 ± 0.182	6.183 ± 0.331	5.871 ± 0.245	5.105 ± 0.165	5.922 ± 0.184	6.371 ± 0.306*
Liver						
Absolute	1.054 ± 0.027	1.120 ± 0.034	1.159 ± 0.038	1.093 ± 0.039	1.051 ± 0.027	1.117 ± 0.040
Relative	32.550 ± 0.756	36.207 ± 1.063*	35.132 ± 0.521	32.419 ± 1.259	33.935 ± 0.650	37.687 ± 0.850**
Lung						
Absolute	0.192 ± 0.008	0.184 ± 0.011	0.201 ± 0.012	0.169 ± 0.006	0.194 ± 0.009	0.187 ± 0.012
Relative	5.936 ± 0.256	6.014 ± 0.462	6.081 ± 0.288	5.033 ± 0.235	6.297 ± 0.341	6.273 ± 0.305
Spleen						
Absolute	0.092 ± 0.003	0.099 ± 0.006	0.103 ± 0.010	0.083 ± 0.005	0.095 ± 0.003	0.097 ± 0.007
Relative	2.858 ± 0.134	3.228 ± 0.232	3.140 ± 0.288	2.460 ± 0.138	3.092 ± 0.160	3.275 ± 0.234
Thymus						
Absolute	0.045 ± 0.003	0.041 ± 0.002	0.047 ± 0.003	0.038 ± 0.003	0.043 ± 0.002	0.042 ± 0.003
Relative	1.378 ± 0.091	1.346 ± 0.077	1.418 ± 0.085	1.135 ± 0.076	1.385 ± 0.084	1.439 ± 0.108

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

APPENDIX D
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE D1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	200 mg/kg	400 mg/kg	800 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	333 ± 6	323 ± 7	307 ± 5**	280 ± 7**
L. Cauda epididymis	0.1689 ± 0.0031	0.1674 ± 0.0033	0.1625 ± 0.0047	0.1382 ± 0.0057**
L. Epididymis	0.4508 ± 0.0070	0.4321 ± 0.0050	0.4334 ± 0.0076	0.3844 ± 0.0111**
L. Testis	1.5084 ± 0.0247	1.5313 ± 0.0199	1.4973 ± 0.0200	1.3929 ± 0.0328**
Spermatid measurements				
Spermatid heads (10^7/g testis)	8.39 ± 0.33	8.30 ± 0.53	9.22 ± 0.41	8.91 ± 0.23
Spermatid heads (10^7/testis)	12.63 ± 0.46	12.71 ± 0.84	13.76 ± 0.54	12.29 ± 0.18
Spermatid count (per 10^{-4} mL suspension)	63.15 ± 2.29	63.55 ± 4.19	68.80 ± 2.69	61.80 ± 0.94
Epididymal spermatozoal measurements				
Motility (%)	83.51 ± 0.81	80.81 ± 0.78	79.58 ± 1.00*	82.33 ± 0.97
Concentration (10^6/g cauda epididymal tissue)	791 ± 27	710 ± 43	700 ± 30	738 ± 33

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Data are presented as mean ± standard error. Differences from the vehicle control group for spermatid measurements and epididymal spermatozoal concentration are not significant by Dunn's test.

TABLE D2
Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	200 mg/kg	400 mg/kg	800 mg/kg
n	10	10	10	10
Necropsy body wt (g)	199 ± 3	189 ± 2	195 ± 3	189 ± 3
Estrous cycle length (days)	4.70 ± 0.13	4.75 ± 0.11	4.70 ± 0.11	4.65 ± 0.31
Estrous stages (% of cycle)				
Diestrus	26.7	20.8	27.5	27.5
Proestrus	21.7	26.7	20.0	20.8
Estrus	23.3	23.3	23.3	22.5
Metestrus	28.3	29.2	29.2	29.2

[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, dosed females do not differ significantly from the vehicle control females in relative length of time spent in the estrous stages.

TABLE D3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	200 mg/kg	400 mg/kg	800 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	39.4 ± 0.9	38.1 ± 1.0	38.5 ± 0.7	35.4 ± 0.7**
L. Cauda epididymis	0.0175 ± 0.0006	0.0172 ± 0.0004	0.0183 ± 0.0007	0.0183 ± 0.0010
L. Epididymis	0.0439 ± 0.0014	0.0435 ± 0.0013	0.0432 ± 0.0010	0.0425 ± 0.0015
L. Testis	0.1154 ± 0.0025	0.1092 ± 0.0034	0.1113 ± 0.0036	0.1084 ± 0.0036
Spermatid measurements				
Spermatid heads (10^7/g testis)	18.80 ± 1.08	19.12 ± 0.89	18.80 ± 1.03	19.31 ± 1.00
Spermatid heads (10^7/testis)	2.16 ± 0.11	2.10 ± 0.14	2.09 ± 0.12	2.07 ± 0.09
Spermatid count (per 10^{-4} mL suspension)	67.38 ± 3.50	65.53 ± 4.28	65.25 ± 3.74	64.83 ± 2.84
Epididymal spermatozoal measurements				
Motility (%)	81.04 ± 0.92	80.45 ± 0.50	81.26 ± 0.77	79.98 ± 0.37
Concentration (10^6/g cauda epididymal tissue)	1,420 ± 106	1,421 ± 71	1,430 ± 52	1,561 ± 100

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

TABLE D4
Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of 2,4-Decadienal[a]

	Vehicle Control	200 mg/kg	400 mg/kg	800 mg/kg
n	10	10	10	9
Necropsy body wt (g)	32.5 ± 0.9	33.9 ± 1.0	31.1 ± 0.9	29.8 ± 1.3
Estrous cycle length (days)	4.00 ± 0.08	4.20 ± 0.11	4.10 ± 0.07	4.33 ± 0.08*
Estrous stages (% of cycle)				
Diestrus	26.7	25.0	25.0	21.3
Proestrus	24.2	25.8	25.0	26.9
Estrus	23.3	24.2	24.2	25.9
Metestrus	25.8	25.0	25.8	25.9

* Significantly different from the vehicle control group ($P \leq 0.05$) by Dunn's test
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group for necropsy body weight are not significant by Dunnett's test. By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in relative length of time spent in the estrous stages.

APPENDIX E
GENETIC TOXICOLOGY

TABLE E1
Mutagenicity of 2,4-Decadienal 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	0.0	111 ± 2.5	114 ± 3.5	127 ± 5.2	113 ± 5.2	125 ± 3.2	124 ± 5.5
	0.3	112 ± 2.8	110 ± 6.4				
	1.0	117 ± 3.1	110 ± 4.5			111 ± 5.4	
	3.0	117 ± 7.8	101 ± 3.1	116 ± 4.0		113 ± 3.7	115 ± 0.9
	10.0	109 ± 2.0	106 ± 4.3	118 ± 2.0	110 ± 3.4	119 ± 4.7	109 ± 8.3
	16.0		80 ± 4.5[d]				
	33.0	92 ± 4.7[d]		118 ± 4.7	112 ± 3.0	106 ± 7.8	113 ± 4.3
	100.0			108 ± 7.5	112 ± 4.9	108 ± 5.0	105 ± 10.1
	333.0			90 ± 2.7[d]	113 ± 2.1		92 ± 9.9[d]
	1,000.0				86 ± 10.0[d]		
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		959 ± 5.8	976 ± 33.0	524 ± 13.0	562 ± 16.6	499 ± 7.2	817 ± 34.8
TA1535	0.0	9 ± 0.9	10 ± 0.9	10 ± 1.2	13 ± 2.0	9 ± 0.6	13 ± 0.9
	0.3	9 ± 0.3	12 ± 0.9				
	1.0	7 ± 0.9	8 ± 0.6			12 ± 0.9	
	3.0	7 ± 0.7	9 ± 0.3	9 ± 1.2		10 ± 0.9	13 ± 1.8
	10.0	8 ± 1.0	11 ± 1.0	9 ± 0.6	13 ± 1.2	10 ± 1.5	16 ± 1.5
	16.0	9 ± 0.9	8 ± 0.3				
	33.0			8 ± 0.9	12 ± 1.2	8 ± 1.2	12 ± 0.3
	100.0			12 ± 3.5	13 ± 0.9	10 ± 0.6	12 ± 1.0
	166.0						9 ± 0.3
	333.0			5 ± 0.7[d]	16 ± 3.0		
	666.0				8 ± 0.6		
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		858 ± 15.0	816 ± 24.4	230 ± 7.5	145 ± 4.4	160 ± 9.5	143 ± 11.1
TA97	0.0	148 ± 5.5	127 ± 4.4	124 ± 11.4	155 ± 6.0	137 ± 5.2	158 ± 6.4
	0.3	143 ± 5.5	122 ± 2.2				
	1.0	153 ± 2.3	146 ± 5.8			137 ± 0.6	
	3.0	147 ± 0.6	134 ± 9.0	137 ± 5.2		146 ± 6.4	149 ± 6.7
	10.0	143 ± 9.5	122 ± 6.5	138 ± 3.0	159 ± 0.3	152 ± 2.2	158 ± 12.1
	16.0	59 ± 22.1[d]	77 ± 6.2[d]				
	33.0			151 ± 1.9	168 ± 13.7	143 ± 9.8	163 ± 12.8
	100.0			143 ± 7.4	162 ± 7.2	120 ± 0.5	161 ± 2.0
	166.0						131 ± 2.4
	333.0			130 ± 7.9	154 ± 21.0		
	666.0				99 ± 5.5		
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		361 ± 21.4	368 ± 14.1	449 ± 14.9	487 ± 12.2	363 ± 21.1	466 ± 18.8

TABLE E1
Mutagenicity of 2,4-Decadienal 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 SRI International (continued)							
TA98	0.0	15 ± 1.7	15 ± 1.2	21 ± 1.9	18 ± 1.7	17 ± 0.3	19 ± 2.6
	0.3	19 ± 2.4	14 ± 0.9				
	1.0	20 ± 1.2	14 ± 2.1			15 ± 1.0	
	3.0	19 ± 3.6	17 ± 0.9	19 ± 3.5		17 ± 1.5	15 ± 0.9
	10.0	20 ± 2.3	15 ± 1.7	20 ± 1.3	15 ± 0.9	17 ± 1.5	22 ± 2.5
	16.0		8 ± 1.0				
	33.0	9 ± 3.8[d,e]		21 ± 0.9	17 ± 2.3	19 ± 4.5	16 ± 3.7
	100.0			18 ± 1.5	16 ± 2.4	16 ± 1.2	19 ± 3.5
	333.0			8 ± 0.9[d]	20 ± 1.2		9 ± 0.9[d]
	1,000.0				7 ± 1.8[d]		
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		337 ± 25.3	327 ± 23.7	418 ± 11.6	424 ± 22.5	350 ± 15.1	407 ± 3.4
Study performed at Environmental Health Research and Testing, Inc.							
TA102	0.0	221 ± 2.6	209 ± 4.4	239 ± 3.2	267 ± 2.3	228 ± 3.5	266 ± 2.3
	0.1	223 ± 3.6	211 ± 3.4				
	0.3	223 ± 3.8	210 ± 5.2				
	1.0	222 ± 2.5	214 ± 4.9	239 ± 3.3	261 ± 3.8	233 ± 4.7	264 ± 4.9
	3.0	225 ± 2.6	207 ± 3.3	237 ± 3.4	262 ± 4.1	233 ± 3.8	266 ± 3.2
	10.0	211 ± 3.9	199 ± 2.4	236 ± 3.2	269 ± 4.1	230 ± 4.6	273 ± 3.7
	33.0			229 ± 4.9	261 ± 4.1	230 ± 3.5	257 ± 4.1
	100.0			224 ± 3.5	255 ± 1.5	227 ± 2.4	267 ± 2.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,077 ± 8.0	1,353 ± 7.8	1,009 ± 8.4	961 ± 6.1	1,017 ± 8.2	980 ± 11.1
TA104	0.0	232 ± 3.8	235 ± 3.5	309 ± 3.8	296 ± 2.7	314 ± 3.3	296 ± 3.5
	0.1	230 ± 4.2	233 ± 3.5				
	0.3	233 ± 3.4	238 ± 3.5				
	1.0	229 ± 3.8	237 ± 4.1	311 ± 4.4	298 ± 5.2	318 ± 3.8	301 ± 4.4
	3.0	216 ± 4.1	237 ± 3.8	312 ± 4.2	300 ± 6.1	310 ± 5.7	295 ± 4.3
	10.0	194 ± 2.3	234 ± 3.5	301 ± 3.2	294 ± 2.6	313 ± 2.0	294 ± 6.5
	33.0			303 ± 4.1	290 ± 4.6	320 ± 3.5	293 ± 3.5
	100.0			287 ± 5.6	240 ± 5.5	283 ± 2.7	240 ± 6.7
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,035 ± 13.9	1,009 ± 5.8	1,068 ± 8.6	1,046 ± 8.5	1,170 ± 7.8	999 ± 11.4

TABLE E1
Mutagenicity of 2,4-Decadienal 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 Environmental Health Research and Testing, Inc. (continued)							
TA100	0.0	141 ± 3.5	128 ± 2.2	121 ± 4.3	139 ± 3.2	137 ± 1.5	144 ± 3.5
	0.1	141 ± 3.0	126 ± 1.9				
	0.3	137 ± 3.2	129 ± 2.9				
	1.0	133 ± 2.8	131 ± 3.1	123 ± 3.6	137 ± 3.5	131 ± 3.5	140 ± 3.2
	3.0	141 ± 4.5	131 ± 3.2	119 ± 4.4	147 ± 4.1	137 ± 3.0	134 ± 4.4
	10.0	140 ± 3.8	104 ± 2.4[d]	119 ± 3.8	133 ± 1.7	133 ± 4.1	133 ± 1.7
	33.0			120 ± 2.7	139 ± 4.1	131 ± 4.1	140 ± 2.7
	100.0			113 ± 3.5	142 ± 3.2	135 ± 4.4	139 ± 4.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		591 ± 5.8	844 ± 37.9	1,017 ± 8.2	859 ± 32.9	906 ± 8.7	457 ± 9.0
TA1535	0.0	21 ± 1.2	23 ± 1.2	14 ± 2.1	19 ± 1.5	15 ± 1.8	18 ± 1.5
	0.1	20 ± 0.3	23 ± 2.3				
	0.3	17 ± 2.3	23 ± 2.2				
	1.0	22 ± 5.3	20 ± 1.9	14 ± 1.7	16 ± 1.7	15 ± 2.2	16 ± 1.2
	3.0	15 ± 0.9	19 ± 2.9	14 ± 1.3	17 ± 0.7	15 ± 2.7	18 ± 1.8
	10.0	3 ± 0.9[d]	17 ± 2.6	14 ± 1.9	17 ± 2.1	13 ± 1.3	16 ± 2.3
	33.0			13 ± 2.3	15 ± 2.0	15 ± 1.7	15 ± 2.0
	100.0			15 ± 2.0	17 ± 1.8	15 ± 2.2	16 ± 1.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		336 ± 3.9	326 ± 4.4	321 ± 6.7	262 ± 6.4	394 ± 2.4	251 ± 6.9
TA97	0.0	101 ± 2.6	100 ± 3.3	121 ± 3.2	133 ± 2.7	130 ± 2.2	124 ± 3.1
	0.1	101 ± 2.1	100 ± 3.9				
	0.3	97 ± 4.0	97 ± 1.8				
	1.0	105 ± 4.0	99 ± 3.2	120 ± 2.7	130 ± 2.6	128 ± 2.8	123 ± 2.6
	3.0	104 ± 2.4	95 ± 1.5	123 ± 2.9	128 ± 3.2	129 ± 2.4	125 ± 2.6
	10.0	98 ± 2.3	90 ± 2.9	116 ± 3.2	133 ± 2.5	127 ± 3.1	122 ± 3.2
	33.0			119 ± 2.4	126 ± 3.2	123 ± 2.6	126 ± 2.6
	100.0			116 ± 2.3	117 ± 2.7	123 ± 3.8	129 ± 2.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		459 ± 13.5	445 ± 4.1	755 ± 5.1	889 ± 6.6	503 ± 24.5	545 ± 4.8

TABLE E1
Mutagenicity of 2,4-Decadienal 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 Environmental Health Research and Testing, Inc. (continued)							
TA98	0.0	24 ± 2.1	23 ± 2.1	36 ± 2.8	32 ± 2.1	26 ± 2.2	31 ± 2.2
	0.1	25 ± 0.9	23 ± 4.7				
	0.3	21 ± 1.5	22 ± 1.5				
	1.0	22 ± 2.5	24 ± 1.2	35 ± 2.7	33 ± 1.2	26 ± 3.2	34 ± 2.0
	3.0	22 ± 1.9	24 ± 3.4	33 ± 2.3	29 ± 1.9	27 ± 2.0	32 ± 2.8
	10.0	24 ± 1.7	19 ± 2.3	32 ± 2.2	30 ± 1.5	29 ± 2.1	33 ± 1.9
	33.0			26 ± 2.0	29 ± 2.6	25 ± 2.3	37 ± 2.1
	100.0			28 ± 1.9	33 ± 2.9	21 ± 1.2	29 ± 3.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		264 ± 7.8	415 ± 7.9	981 ± 7.2	556 ± 4.0	822 ± 8.4	425 ± 8.4

[a] The detailed protocol is presented by Zeiger *et al*. (1992). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), 4-nitro-*o*-phenylenediamine (TA98), mitomycin-C (TA102), and methyl methanesulfonate (TA104). The positive control for metabolic activation with all strains was 2-aminoanthracene, and 2-aminoanthracene or sterigmatocystin was used for TA102.
[d] Slight toxicity
[e] Precipitate on plate

TABLE E2
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with 2,4-Decadienal by a Single Intraperitoneal Injection[a]

	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]
Corn oil[d]		5	0.30 ± 0.20	
2,4-Decadienal	100	5	1.50 ± 0.52	0.0023
	200	5	1.50 ± 0.42	0.0023
	400	4	1.88 ± 0.52	0.0004
	600	4	0.63 ± 0.13	0.1520
			P=0.294[e]	
Cyclophosphamide[f]	25	5	7.50 ± 2.77	0.0000

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Shelby *et al*. (1993); bone marrow was sampled 24 hours after the intraperitoneal injection. PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control. Dosed group values are significant at P≤0.006; positive control value is significant at P≤0.05 (ILS, 1990)
[d] Vehicle control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test; significant at P≤0.025 (ILS, 1990)
[f] Positive control

TABLE E3
Induction of Micronuclei in Bone Marrow and Peripheral Blood Polychromatic Erythrocytes of Male Mice Treated with 2,4-Decadienal by Intraperitoneal Injection[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]
Trial 1: Bone Marrow[d]				
Corn oil[e]		5	1.20 ± 0.37	
2,4-Decadienal	25	5	0.60 ± 0.19	0.9214
	50	5	1.00 ± 0.22	0.6652
	100	5	1.40 ± 0.24	0.3473
	200	4	1.50 ± 0.35	0.2918
			P=0.084[f]	
Cyclophosphamide[g]	25	5	3.00 ± 0.42	0.0027
Trial 2: Bone Marrow[h]				
Corn oil		5	0.60 ± 0.10	
2,4-Decadienal	400	5	0.70 ± 0.30	0.3907
	600	5	2.10 ± 0.29	0.0019
			P=0.003	
Cyclophosphamide	25	5	3.50 ± 0.71	0.0000
Trial 3: Peripheral Blood[i]				
Corn oil		4	2.13 ± 0.55	
2,4-Decadienal	400	5	3.30 ± 0.58	0.0683
	600	5	3.60 ± 0.51	0.0348
			P=0.034	
Cyclophosphamide	25	4	10.63 ± 0.72	0.0000

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Shelby *et al*. (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control. Dosed group values are significant at P≤0.006 (Trial 1) or P≤0.012 (Trials 2 and 3); positive control values are significant at P≤0.05 (ILS, 1990)
[d] Three intraperitoneal injections at 24-hour intervals with bone marrow sampling 24 hours after the third injection
[e] Vehicle control
[f] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test; significant at P≤0.025 (ILS, 1990)
[g] Positive control
[h] One intraperitoneal injection followed by bone marrow sampling 48 hours later
[i] One intraperitoneal injection followed by peripheral blood analysis 48 hours later (same animals as were used in Trial 2)

TABLE E4
Frequency of Micronuclei in Peripheral Blood Normochromatic Erythrocytes of Mice Following Treatment with 2,4-Decadienal by Gavage for 3 Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]
Male				
Corn oil[d]		10	1.80 ± 0.29	
2,4-Decadienal	50	10	2.30 ± 0.30	0.2172
	100	10	2.50 ± 0.22	0.1426
	200	10	2.50 ± 0.52	0.1426
	400	10	2.10 ± 0.43	0.3153
	800	10	2.70 ± 0.47	0.0896
			P=0.198[e]	
Female				
Corn oil		10	2.00 ± 0.26	
2,4-Decadienal	50	10	1.70 ± 0.30	0.6892
	100	9	2.00 ± 0.44	0.5000
	200	10	1.50 ± 0.31	0.8012
	400	10	1.80 ± 0.36	0.6273
	800	9	2.11 ± 0.39	0.4329
			P=0.344	

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

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of 2,4-Decadienal

2,4-Decadienal was obtained from Lancaster Synthesis (Windham, NH) in one lot (90000694). Lot 90000694 was used in the 2-week and 3-month studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Research Triangle Institute (Research Triangle Park, NC) and the study laboratories, Microbiological Associates, Inc. (Rockville, MD), and Southern Research Institute (Birmingham, AL). Reports on analyses performed in support of the 2,4-decadienal studies are on file at the National Institute of Environmental Health Sciences.

Lot 90000694 of the chemical, a pale yellow liquid, was identified as 2,4-decadienal by the analytical chemistry laboratory using infrared and nuclear magnetic resonance spectroscopy. Identity was confirmed by the study laboratories using infrared and nuclear magnetic resonance (Southern Research Institute) spectroscopy. Infrared spectra were consistent with the structure of 2,4-decadienal. Nuclear magnetic resonance spectra were consistent with the structure of 2,4-decadienal and with literature spectra (*Aldrich*, 1983). The infrared and nuclear magnetic resonance spectra are presented in Figures F1 and F2.

The purity of lot 90000694 was determined by the analytical chemistry laboratory using gas chromatography with flame ionization detection by system A. Purity was confirmed by the study laboratories using gas chromatography by systems A and B.

A) J&W SE-30 column (30 m × 0.32 mm inner diameter, 0.25-µm film thickness; J&W Scientific, Folsom, CA), helium carrier gas at 1 mL/minute and an oven temperature program of 50° C to 250° C at 5° C per minute.

B) J&W DB-17 capillary column (15 m × 0.25 mm inner diameter, 0.25-µm film thickness; J&W Scientific, Folsom, CA) with an oven temperature program of 50° C to 250° C at 5° C per minute.

Gas chromatography by system A indicated a purity of approximately 94% and one major peak and five impurities with a combined area of approximately 5.7% relative to the major peak area; a reanalysis using system A indicated a purity of approximately 98.8% relative to a reference standard that had been stored at –20° C under nitrogen. Gas chromatography by system B indicated a purity of 93.7% with six impurities.

To ensure stability, the bulk chemical was stored at approximately 5° C, protected from light, in the shipping container under nitrogen. Stability was monitored during the 2-week and 3-month studies using gas chromatography as described above. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing 2,4-decadienal with corn oil to give the required concentrations (Table F1). The dose formulations were stored at approximately 5° C, protected from light, for up to 35 days in amber bottles.

Homogeneity studies of 5 and 160 mg/mL dose formulations were performed by Southern Research Institute with high-performance liquid chromatography (HPLC) using ultraviolet detection (254 nm), a Zorbax C-8 column (250 mm × 4.6 mm, 5 µm particle size; Dupont, Wilmington, DE), and a mobile phase of acetonitrile:water (50:50)

at a flow rate of 1.5 mL/minute. Stability studies of a 4.5 mg/mL dose formulation were also performed by the analytical chemistry laboratory using the HPLC system described above. Homogeneity was confirmed, and stability was confirmed for at least 35 days for dose formulations stored in sealed glass vials at refrigerator (2° to 5° C) or room (23° to 28° C) temperature and for 3 hours for dose formulations exposed to light and air at room temperature.

Periodic analyses of the dose formulations of 2,4-decadienal were conducted by the analytical chemistry laboratory (2-week studies) and Southern Research Institute (3-month studies) using HPLC by the system described above. During the 2-week studies, the dose formulations were analyzed once (Table F2). Animal room samples of these dose formulations were also analyzed; all 10 of the animal room samples were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed (Table F3). All 15 of the dose formulations used in the studies for each species were within 10% of the target concentrations, with no value greater than 107% of the target concentration; all 15 of the animal room samples for each species were within 10% of the target concentrations as well. Periodic analyses of the corn oil vehicle by the study laboratories demonstrated peroxide concentrations within the acceptable limit of 3 mEq/kg.

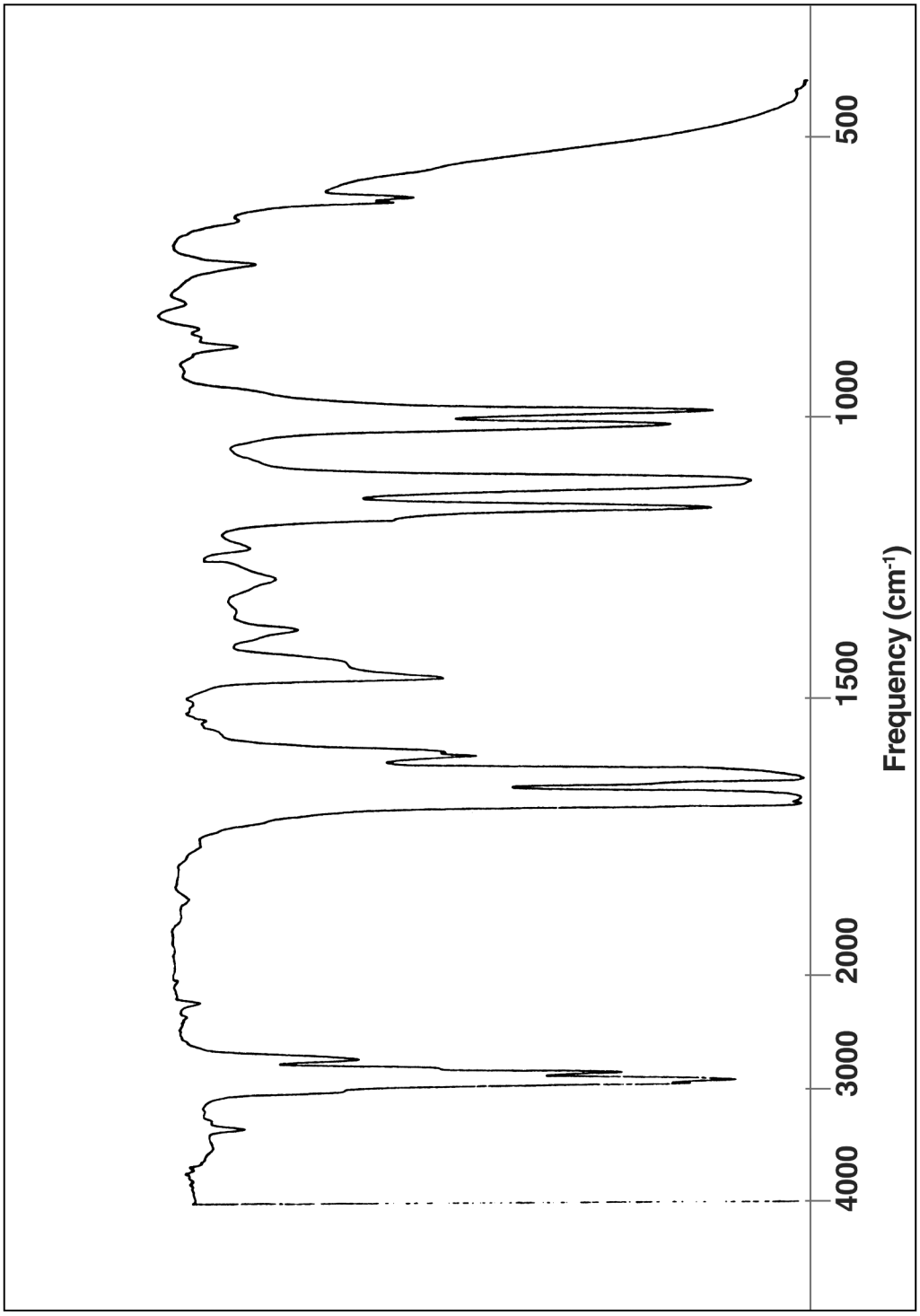

Figure F1
Infrared Absorption Spectrum of 2,4-Decadienal

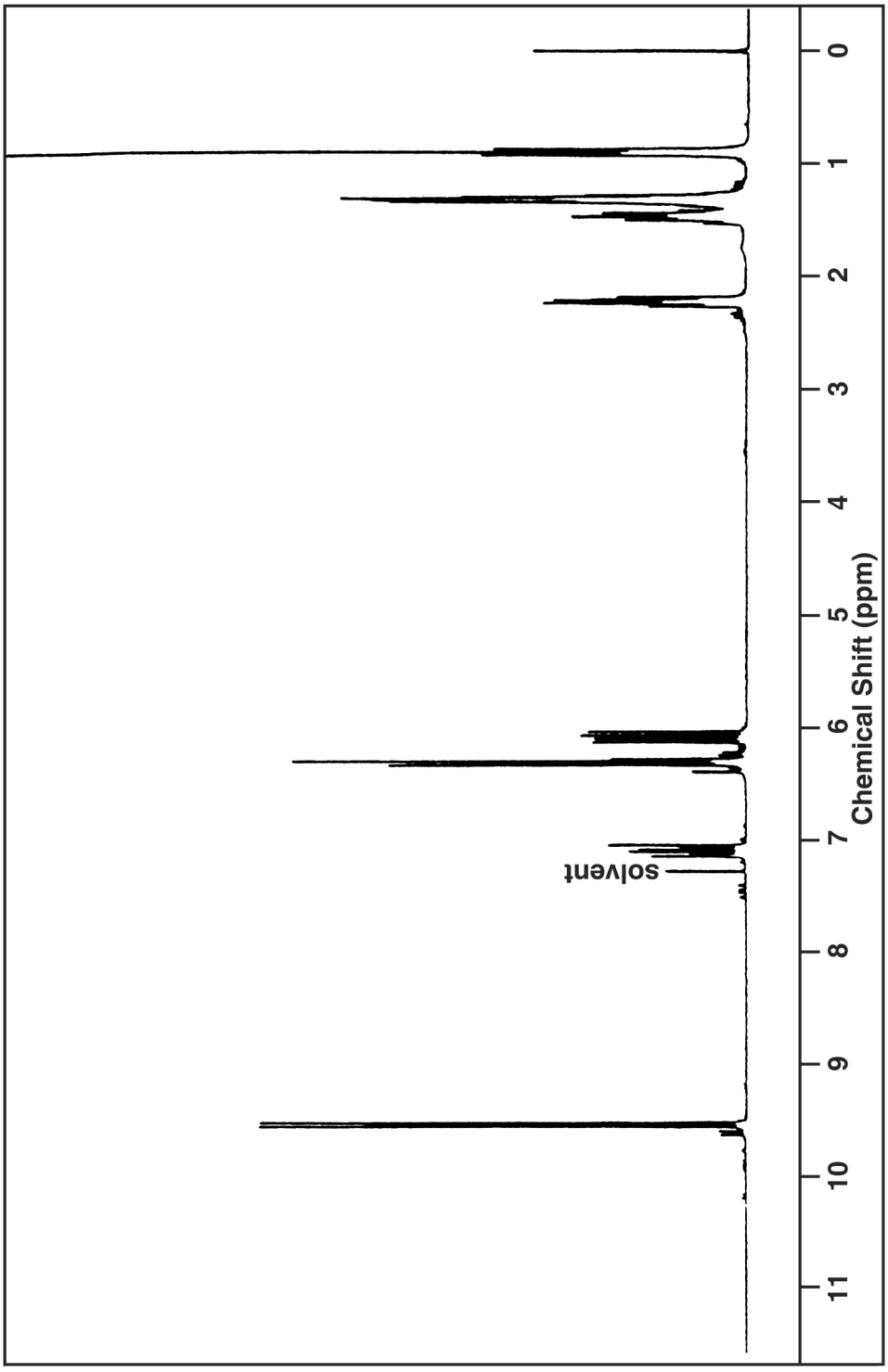

Figure F2
Nuclear Magnetic Resonance Spectrum of 2,4-Decadienal

TABLE F1
Preparation and Storage of Dose Formulations in the Gavage Studies of 2,4-Decadienal

2-Week Studies	3-Month Studies
Preparation	
2,4-Decadienal was mixed with corn oil to give the required concentrations. Dose formulations were prepared once.	2,4-Decadienal was mixed with corn oil to give the required concentrations. Dose formulations were prepared at 3- to 4-week intervals.
Chemical Lot Number	
90000694	90000694
Maximum Storage Time	
35 days	35 days
Storage Conditions	
Stored refrigerated under nitrogen	Stored in amber glass bottles at approximately 5° C
Study Laboratory	
Microbiological Associates, Inc. (Bethesda, MD)	Southern Research Institute (Birmingham, AL)

TABLE F2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Gavage Studies of 2,4-Decadienal

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
November 6, 1995	December 12-13, 1995[b]	18	18.6	+3
		53.2	55.3	+4
		160	170	+6
		480	503	+5
		720	753	+5
Mice				
November 6, 1995	December 12-13, 1995[b]	18	18.9	+5
		53.2	55.5	+4
		160	169	+6
		480	442[c]	-8
		720	727	+1

[a] Results of triplicate analyses. Dosing volume=2.5 mL/kg (18 to 480 mg/mL) or 5.0 mL/kg (720 mg/mL); 18 mg/mL=45 mg/kg, 53.2 mg/mL=133 mg/kg, 160 mg/mL=400 mg/kg, 480 mg/mL=1,200 mg/kg, 720 mg/mL=3,600 mg/kg
[b] Animal room samples
[c] Result of four samples

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of 2,4-Decadienal

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
July 23, 1996	July 24-25, 1996	10	10.1	+1
		20	20.1	0
		40	40.1	0
		80	80.6	+1
		160	159	0
	August 9-10, 1996[b]	10	10.1	+1
		20	20.2	+1
		40	40.7	+2
		80	81.5	+2
		160	163	+2
September 17, 1996	September 18-19, 1996	10	10.3	+3
		20	21	+5
		40	42.1	+5
		80	84.4	+6
		160	171	+7
	October 21-22, 1996[b]	10	10.4	+4
		20	21	+5
		40	42	+5
		80	83.7	+5
		160	168	+5
October 15, 1996	October 15-16, 1996	10	10.5	+5
		20	21.2	+6
		40	42.6	+6
		80	84.5	+6
		160	169	+6
	November 5-6, 1996[b]	10	10.4	+4
		20	20.7	+3
		40	41.4	+4
		80	82.9	+4
		160	165	+3

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of 2,4-Decadienal

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
July 23, 1996	July 24-25, 1996	5	5.08	+2
		10	10.1	+1
		20	20.1	0
		40	40.1	0
		80	80.6	+1
	August 9-10, 1996[b]	5	5.02	0
		10	10.2	+2
		20	20.3	+2
		40	40.7	+2
		80	81.9	+2
September 17, 1996	September 18-19, 1996	5	5.11	+2
		10	10.3	+3
		20	21	+5
		40	42.1	+5
		80	84.4	+6
	October 21-22, 1996[b]	5	5.3	+6
		10	10.4	+4
		20	21	+5
		40	41.9	+5
		80	83.6	+4
October 15, 1996	October 15-16, 1996	5	5.95[c]	+19
		10	10.5	+5
		20	21.2	+6
		40	42.6	+6
		80	84.5	+6
	November 5-6, 1996[b]	5	5.01	0
		10	10.3	+3
		20	20.8	+4
		40	41.3	+3
		80	82.3	+3
October 16, 1996	October 17, 1996	5	5.05[d]	+1

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 10 mg/mL=50 mg/kg, 20 mg/mL=100 mg/kg, 40 mg/mL=200 mg/kg, 80 mg/mL=400 mg/kg; 160 mg/mL=800 mg/kg; for mice, dosing volume=10 mL/kg; 5 mg/mL=50 mg/kg, 10 mg/mL=100 mg/kg, 20 mg/mL=200 mg/kg, 40 mg/mL=400 mg/kg, 80 mg/mL=800 mg/kg
[b] Animal room samples
[c] Remixed; not used in study
[d] Results of remix

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