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

NTP Technical Report on the Comparative Toxicity Studies of Allyl Acetate, Allyl Alcohol, and Acrolein (CAS Nos. 591-87-7, 107-18-6, and 107-02-8) Administered by Gavage to F344/N Rats and B6C3F$_1$ Mice

NTP TOX 48

JULY 2006

National Toxicology Program
Toxicity Report Series
Number 48

NTP Technical Report
on the Comparative Toxicity Studies of

Allyl Acetate, Allyl Alcohol, and Acrolein

(CAS Nos. 591-87-7, 107-18-6, and 107-02-8)

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

July 2006

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

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Toxicity Study Report series began in 1991. The studies described in the Toxicity Study Report series are designed and conducted to characterize and evaluate the toxicologic potential of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in the Toxicity Study Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's toxic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

NTP Toxicity Study Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*) or in hardcopy upon request from the NTP Central Data Management group at *cdm@niehs.nih.gov* or (919) 541-3419.

National Toxicology Program
Toxicity Report Series
Number 48

NTP Technical Report
on the Comparative Toxicity Studies of

Allyl Acetate, Allyl Alcohol, and Acrolein

(CAS Nos. 591-87-7, 107-18-6, and 107-02-8)

Administered by Gavage
to F344/N Rats and B6C3F_1 Mice

Rick D. Irwin, Ph.D., Study Scientist

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

NIH Publication No. 06-4413

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

CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

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

Battelle Columbus Laboratories
Conducted studies, evaluated pathology findings

M.R. Hejtmancik, Ph.D., Principal Investigator
M.J. Ryan, D.V.M., Ph.D.
A.W. Singer, D.V.M.
J.D. Toft, II, D.V.M., M.S.

Experimental Pathology Laboratories, Inc.
Provided pathology review

J.F. Hardisty, D.V.M., Principal Investigator
S. Botts, M.S., D.V.M., Ph.D.

Constella Group, Inc.
Provided statistical analyses

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

NTP Pathology Working Group
Evaluated slides and prepared pathology report of allyl acetate (January 8, 1997)

J.C. Seely, D.V.M., Chairperson
PATHCO, Inc.
S. Botts, M.S., D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
J.R. Leininger, D.V.M., Ph.D.
National Toxicology Program
J. Mahler, D.V.M.
National Toxicology Program
A. Nyska, D.V.M.
National Toxicology Program
A. Radovsky, D.V.M., Ph.D.
National Toxicology Program
D. Wolf, D.V.M., Ph.D.
Chemical Industry Institute of Toxicology

Evaluated slides and prepared pathology reports of allyl alcohol and acrolein (March 4, 1997)

J.C. Seely, D.V.M., Chairperson
PATHCO, Inc.
S. Botts, M.S., D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
J.R. Leininger, D.V.M., Ph.D.
National Toxicology Program
J. Mahler, D.V.M.
National Toxicology Program
A. Radovsky, D.V.M., Ph.D.
National Toxicology Program
D. Wolf, D.V.M., Ph.D.
Chemical Industry Institute of Toxicology

Biotechnical Services, Inc.
Prepared Toxicity Study Report

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

PEER REVIEW

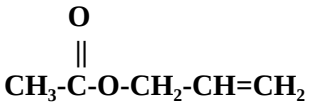
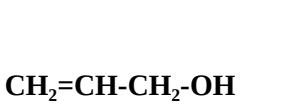
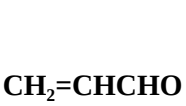
The draft report on the toxicity studies of allyl acetate, allyl alcohol, and acrolein was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the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

James P. Kehrer, Ph.D.
College of Pharmacy
University of Texas
Austin, TX

Mary Vore, Ph.D.
Graduate Center for Toxicology
University of Kentucky
Lexington, KY

CONTENTS

ABSTRACT

	Allyl Acetate	Allyl Alcohol	Acrolein
			
CAS Number:	591-87-7	107-18-6	107-02-8
Molecular Weight:	100.12	58.08	56.06
Synonyms and Trade Names:	allyl alcohol, acetate; 3-acetoxy-1-propene; 3-acetoxy-propene	allylic alcohol; 3-hydroxypropene; 1-propenol-3; 2-propenol; 2-propenyl alcohol; Shell unkrautted A; Weed Drench; vinyl carbinol	acraldehyde; allyl aldehyde; 2-propenal

Allyl acetate, allyl alcohol, and acrolein are used in the manufacture of detergents, plastics, pharmaceuticals, and chemicals and as agricultural agents and food additives. Male and female F344/N rats and B6C3F$_1$ mice received allyl acetate, allyl alcohol, or acrolein by gavage for 14 weeks. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Drosophila melanogaster*, cultured Chinese hamster ovary cells, rat bone marrow erythrocytes, and mouse peripheral blood erythrocytes.

Groups of 10 male and 10 female rats were administered 0, 6, 12, 25, 50, or 100 mg allyl acetate/kg body weight, 0, 1.5, 3, 6, 12, or 25 mg/kg allyl alcohol, or 0, 0.75, 1.25, 2.5, 5, or 10 mg/kg acrolein in 0.5% methylcellulose by gavage, 5 days per week for 14 weeks. Groups of 10 male and 10 female mice were administered 0, 8, 16, 32, 62.5, or 125 mg/kg allyl acetate, 0, 3, 6, 12, 25, or 50 mg/kg allyl alcohol, or 0, 1.25, 2.5, 5, 10, or 20 mg/kg acrolein in 0.5% methylcellulose by gavage, 5 days per week for 14 weeks.

In the allyl acetate rat study, all males and females in the 100 mg/kg groups died or were killed moribund by day 8; there were no other deaths. In the allyl alcohol study, all rats survived to the end of the study except one 6 mg/kg female. In the acrolein rat study, eight males and eight females in the 10 mg/kg groups died by week 9 of the study. Two males in the 2.5 and 5 mg/kg groups and one or two females in the 1.25, 2.5, and 5 mg/kg groups also died early; two of these deaths were gavage accidents. In the allyl acetate mouse study, all males and females in the 125 mg/kg group died during the first week of the study. All other early deaths, except five 62.5 mg/kg males and one 32 mg/kg female, were gavage accidents. In the allyl alcohol mouse study, one 50 mg/kg female died due to a gavage accident; all other

animals survived to the end of the study. In the acrolein mouse study, all males and females administered 20 mg/kg died during the first week of the study. All other early deaths, except one male and one female administered 10 mg/kg, were unrelated to chemical administration.

The concentration of 3-hydroxypropyl mercapturic acid (3-HPM) in the urine of rats and mice was determined after the first dose of chemical and at the end of the 14-week study. At both time points, the concentrations of 3-HPM in the urine of animals that received allyl acetate or allyl alcohol increased linearly with dose. In animals dosed with acrolein, the concentrations of 3-HPM exhibited a nonlinear increase with dose at the first time point. At the end of the study, the concentration of 3-HPM in the urine of animals dosed with acrolein was linear with dose except at the highest concentration administered. Since urine volumes were not recorded during the urine collection, complete quantitation of these data was not possible.

The final mean body weights and mean body weight gains of male rats administered 12 or 50 mg/kg allyl acetate and of male and female rats administered 10 mg/kg acrolein were significantly less than those of the vehicle controls. The mean body weight gain of male mice in the 50 mg/kg group in the allyl alcohol study was also less than that of the vehicle controls. Final mean body weights and mean body weight gains of dosed female rats and male and female mice in the allyl acetate studies, male and female rats and female mice in the allyl alcohol studies, and male and female mice in the acrolein studies were generally similar to those of the respective vehicle controls.

Clinical findings related to allyl acetate administration included pallor, eye or nasal discharge, ruffled fur, lethargy, diarrhea, and thinness among rats in the 100 mg/kg groups and lethargy, abnormal breathing, thinness, and ruffled fur among mice that died early. In the acrolein study, clinical findings included abnormal breathing, eye or nasal discharge, ruffled fur, thinness, and lethargy in rats in the 10 mg/kg groups.

The liver weights of male rats administered 25 mg/kg allyl alcohol, female rats administered 50 mg/kg allyl acetate or 5 or 10 mg/kg acrolein, and male mice administered 10 mg/kg acrolein were significantly greater than those of the vehicle controls. Female rats administered 10 mg/kg acrolein had significantly lower absolute and relative thymus weights than did the vehicle controls.

Female rats administered 25 mg/kg allyl alcohol spent more time in diestrus and less time in metestrus than the vehicle controls. The estrous cycles of female mice dosed with 16 or 32 mg/kg allyl acetate were significantly longer than that of the vehicle controls.

Gross lesions related to allyl acetate treatment were observed in the liver, forestomach, and thorax/abdomen of male and female rats in the 100 mg/kg groups. Microscopically, the incidences of forestomach squamous epithelial hyperplasia were significantly increased in male rats administered 12 mg/kg or greater, female rats administered 25 or 50 mg/kg, male mice administered 32 or 62.5 mg/kg, and female mice administered 16, 32, or 62.5 mg/kg. Forestomach necrosis, hemorrhage, and inflammation were present in most rats in the 100 mg/kg groups, and the incidence of hemorrhage in 125 mg/kg male mice was increased; male mice in the 62.5 and 125 mg/kg groups and 125 mg/kg female mice had significantly increased incidences of glandular stomach hemorrhage. Increased incidences of several liver lesions occurred in male or female rats administered 50 or 100 mg/kg, and to a lesser extent in 25 mg/kg rats, 62.5 mg/kg male mice, and 125 mg/kg male and female mice.

Bone marrow hyperplasia, hemorrhage or depletion in the mediastinal, mandibular, and mesenteric lymph nodes, hemorrhage and necrosis of the thymus, and hematopoietic cell proliferation of the red pulp were also observed in 100 mg/kg rats. Increased incidences of necrosis in the mandibular and mesenteric lymph nodes, spleen, and thymus were observed in 62.5 and 125 mg/kg mice.

Male and female rats administered 6 mg/kg allyl alcohol or greater and male and female mice administered 12 mg/kg allyl alcohol or greater had significantly increased incidences of squamous hyperplasia of the forestomach epithelium. Female rats in the 25 mg/kg group had significantly increased incidences of bile duct hyperplasia and periportal hepatocyte hypertrophy in the liver. Incidences of portal cytoplasmic vacuolization were significantly increased in 50 mg/kg male mice and female mice in the 25 and 50 mg/kg groups.

Gross lesions related to acrolein treatment were observed in the forestomach and glandular stomach of male and female rats in the 10 mg/kg groups and 20 mg/kg female mice. Microscopically, the incidences of squamous hyperplasia of the forestomach epithelium were significantly increased in male rats in the 5 and 10 mg/kg groups, female rats administered 2.5 mg/kg or greater, and male and female mice administered 2.5, 5, or 10 mg/kg. Male and female rats in the 10 mg/kg groups and 20 mg/kg male and female mice had significantly increased incidences of glandular stomach hemorrhage. Female mice in the 20 mg/kg group also had significantly increased incidences of glandular stomach inflammation and epithelial necrosis.

Allyl acetate was mutagenic in *S. typhimurium* strains TA100 and TA1535, in the absence of S9 activation. With S9, no mutagenicity was detected in these two strains; negative results were obtained in strains TA97 and TA98, with and without S9. Allyl alcohol was not mutagenic in four strains of *S. typhimurium*, with or without S9 metabolic activation. Acrolein, tested in a preincubation protocol, was weakly mutagenic in *S. typhimurium* strain TA100 in the presence of 10% induced rat liver S9. Equivocal results were obtained in strains TA100 and TA1535 with 10% induced hamster liver S9. Negative results were obtained with TA97, TA98, and TA1538 under all test conditions, and acrolein gave negative results in all four *S. typhimurium* strains tested for mutation induction under a vapor protocol. No induction

of micronuclei was noted in bone marrow erythrocytes of male rats administered allyl acetate by gavage three times at 24-hour intervals. No significant increases in micronucleated erythrocytes were noted in bone marrow samples from male rats administered allyl alcohol by intraperitoneal injection for 3 days. A small, but significant increase in the frequency of micronucleated normochromatic erythrocytes was observed in the peripheral blood of female mice administered allyl acetate by gavage for 14 weeks; no increase was observed in male mice. No increases in the frequencies of micronucleated normochromatic erythrocytes were observed in the peripheral blood of male or female mice administered allyl alcohol or acrolein by gavage for 14 weeks. Acrolein induced sister chromatid exchanges in cultured Chinese hamster ovary cells in the absence, but not the presence, of S9; it did not induce chromosomal aberrations, with or without S9. Results of three independent *Drosophila melanogaster* sex-linked recessive lethal tests in which acrolein was administered to adult flies via feeding or injection and to larvae via feeding were negative.

INTRODUCTION

PHYSICAL PROPERTIES

The chemical and physical properties of allyl acetate and allyl alcohol are presented in Table 1.

TABLE 1

	Allyl Acetate[a]	Allyl Alcohol[b]
CAS Registry Number	591-87-7	107-18-6
Chemical Abstracts Name	Acetic acid, 2-propenyl ester (9CI); acetic acid, allyl ester (8CI)	2-Propen-1-ol (9CI)
Synonyms and Trade Names	Allyl alcohol, acetate; 3-acetoxy-1-propene; 3-acetoxy-propene	Allylic alcohol; 3-hydroxypropene; 1-propenol-3; 2-propenol; 2-propenyl alcohol; Shell unkrautted A; Weed Drench; vinyl carbinol
Molecular Formula	$C_5H_8O_2$	C_3H_6O
Structure	$CH_3\text{-}\overset{\overset{O}{\|}}{C}\text{-}O\text{-}CH_2\text{-}CH{=}CH_2$	$CH_2{=}CH\text{-}CH_2\text{-}OH$
Molecular Weight	100.12	58.08
Description	Colorless liquid with an acrid odor at high levels	Colorless liquid with a pungent mustard-like odor
Boiling Point	103.5° C at 760 mm Hg	96°-97° C
Melting Point	22° C	-129° C
Solubility	Slightly soluble in water; soluble in acetone; very soluble with alcohol and ether	Miscible with water, alcohol, chloroform, ether, and petroleum ether
Stability	Acrid smoke and irritating fumes are emitted when heated to decomposition; can be ignited under ambient conditions	Stable at ordinary temperatures and pressures; polymerizes and forms a thick syrup upon storage for several years
Reactivity	Reacts with oxidizing materials	
Density	0.9276 g/mL	0.8540 (at 20° C/4° C)
Vapor Density	3.45	20 mm at 20° C; 32 mm at 30° C
Refractive Index	1.4049	
Flash Point	21° C	70° F (open cup), 75° F (closed cup)

[a] Sandmeyer and Kirwin, 1981; Sax and Lewis, 1987, 1989; Weast, 1989; or HSDB, 2001
[b] Verschueren, 1983; Weiss, 1986; *Merck Index*, 1989; or Weast, 1989

PRODUCTION AND USE

Allyl acetate is an important intermediate in the synthesis of many industrial chemicals and has several industrial applications. It is used in the production of fire-resistant plastics and resins; in hair conditioning formulations; in low-phosphate detergents as a detergent builder, where it replaces sodium tripolyphosphate; in the synthesis of 1,4-butanediol, another industrially important intermediate; and in the manufacture of ester-containing siloxanes for brake fluids. Although allyl acetate is available from many chemical suppliers, domestic production has not been reported recently.

Allyl alcohol is an important industrial chemical. The direct oxidation of allyl alcohol to glycerol by peroxide is the most widely used method of glycerol production, and this method consumes approximately 50 kilotons of allyl alcohol annually in the United States. Allyl alcohol is also used in the commercial synthesis of acrolein; in the production of various allyl esters, including diallyl phthalate; in the production of plastic lenses, silicone surfactants, and pharmaceuticals; and as a solvent. In addition, allyl alcohol, as well as several allyl esters, has been used as a flavoring agent.

METABOLISM

Silver and Murphy (1978) studied the toxicity of allyl acetate and several other esters of allyl alcohol. Hepatotoxicity in rats pretreated with carboxyl esterase inhibitors was compared with that in rats that had not been pretreated. Hydrolysis of allyl acetate by liver homogenates from rats pretreated with triorthotolyl phosphate (TOTP) was inhibited 97.7% compared to hydrolysis by homogenates from control rats. Rats pretreated with TOTP prior to receiving 60 or 150 mg allyl acetate/kg body weight by gavage had significantly lower alanine transaminase (ALT) activities than rats that did not receive TOTP. DEF (S,S,S-tributylphosphoro-trithioate), also a well-known esterase inhibitor, produced results similar to those seen with TOTP. Interestingly, rats pretreated with pyrazole, an inhibitor of alcohol dehydrogenase, exhibited no increase in serum ALT activity after administration of 90 mg/kg allyl acetate (Silver and Murphy, 1978). These results indicated that the hepatotoxicity associated with administration of allyl acetate (and other esters of allyl alcohol) is due to the release of allyl alcohol as a result of the rapid hydrolysis of these esters in liver, blood, and other tissues.

The toxicity of allyl alcohol has been studied extensively, and its hepatotoxicity has been documented in numerous studies (Badr, 1991). Administration of hepatotoxic doses of allyl alcohol causes necrosis in periportal regions of the liver lobule in rodents; however, the ultimate toxicant appears to be acrolein formed by the oxidation of allyl alcohol. The importance of liver alcohol dehydrogenase (ADH) to the toxicity of allyl alcohol has been demonstrated in several studies; prior treatment of rats with ADH inhibitors significantly reduces the hepatotoxicity of allyl alcohol (Reid, 1972).

A strain of deer mice devoid of alcohol dehydrogenase activity due to a genetic defect in the ADH gene exhibited no detectable toxic response as measured by histopathology and serum sorbitol dehydrogenase and serum glutamic oxaloacetic transaminase activities after the mice received doses of allyl alcohol that caused marked increases in serum enzyme activity and periportal necrosis of the liver in a strain of deer mice that express normal levels of ADH activity (Belinsky *et al.*, 1985). Moreover, the age-associated increase in ADH activity observed in male F344 rats correlates well with the age-associated increase in allyl alcohol hepatotoxicity. The lack of an age-associated increase in ADH activity in female F344 rats also correlates with the lack of an age-associated increase in allyl alcohol hepatotoxicity in females (Rikans and Moore, 1987). Preventing the detoxification of acrolein also enhances the hepatotoxicity of allyl alcohol. Prior treatment of F344 rats with aldehyde dehydrogenase inhibitors significantly enhances allyl alcohol hepatotoxicity (Rikans, 1987). While there appears to be a consensus that acrolein is responsible for the hepatotoxicity of allyl alcohol, the mechanism by which acrolein is cytotoxic to hepatocytes is an active area of investigation, and mechanisms involving lipid peroxidation and oxygen radical formation have been proposed (Badr, 1991; Adams and Klaidman, 1993).

In addition to being oxidized to acrylic acid, acrolein is a good substrate for glutathione transferase (Berhane and Mannervik, 1990), and glutathione conjugation is considered a major route of acrolein detoxification as evidenced by the presence of S-(3-hydroxypropyl) mercapturic acid and S-(2-carboxyethyl) mercapturic acid in the urine of rats administered acrolein, allyl alcohol, allyl chloride, allyl amine, or allyl bromide (Kaye, 1973; Sanduja *et al.*, 1989). Acrolein is also capable of reacting with sulfhydryl groups nonenzymatically via a Michael addition, and reaction with critical intracellular sulfhydryl groups has been proposed as a component of the cytotoxicity of acrolein (Cooper *et al.*, 1992; Kehrer and Biswal, 2000).

Administration of the 1:1 adduct of glutathione with acrolein (carbon-3) in rats produced nephrotoxicity that could be prevented by pretreatment with acivicin, a γ-glutamyl transpeptidase inhibitor, but was unaffected by pretreatment with inhibitors of ADH, aldehyde dehydrogenase, or probenecid, a renal organic anion transporter inhibitor (Horvath *et al.*, 1992). Moreover, S-*n*-propylglutathione, which has the same carbon skeleton but lacks a terminal aldehyde function on the propyl group, was not nephrotoxic.

The metabolism of acrolein in rats has been examined in detail by Parent *et al.* (1996a, 1998). Following oral (gavage) administration, the major metabolites identified in the urine of Sprague-Dawley rats were malonic acid, 3-hydroxyproprionic acid, *N*-acetyl-S-2-carboxy-2-hydroxyethylcysteine, *N*-acetyl-S-3-hydroxypropylcysteine, and *N*-acetyl-S-2-carboxyethylcysteine (Figure 1). Therefore, the main pathway appears to be Michael addition of glutathione to the activated double bond, followed by processing to the mercapturic acid, which is excreted in the urine after either oxidation or reduction of the aldehyde, with reduction predominating. These compounds have previously been reported as metabolites of acrolein (Kaye, 1973; Draminski *et al.*, 1983; Sanduja *et al.*, 1989; Linhart *et al.*, 1996).

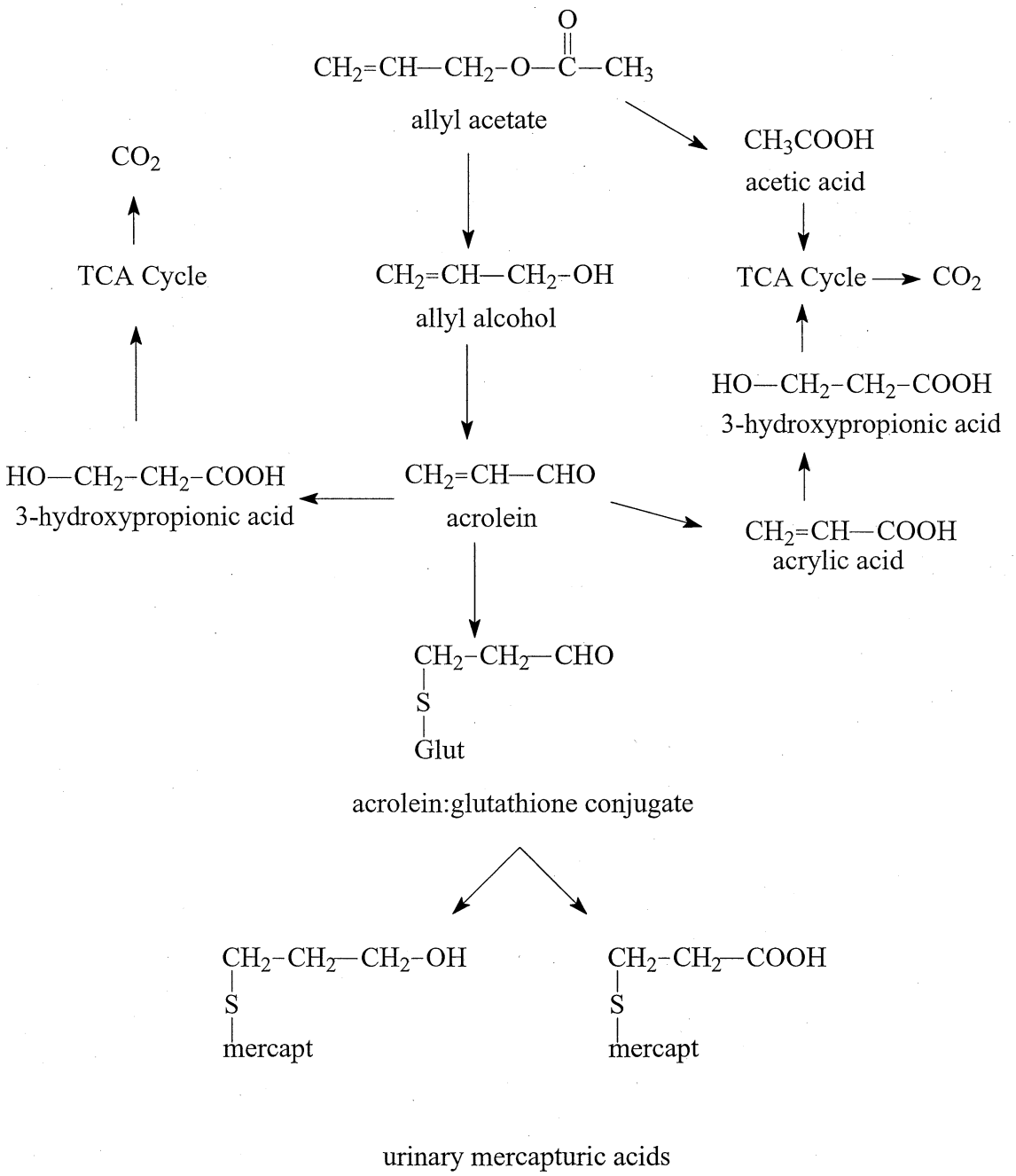


FIGURE 1
Metabolism of Allyl Acetate, Allyl Alcohol, and Acrolein

The presence of malonic acid and 3-hydroxyproprionic acid is the result of Michael addition of water to acrolein to form 3-hydroxypropionaldehyde, followed by oxidation to malonic and ultimately oxalic acid. The addition of water to acrolein is a well-studied nonenzymatic process that can occur in the gastrointestinal tract, most likely by the gut flora, as these metabolites are not present in the urine of intravenously dosed rats (Parent *et al.*, 1998).

TOXICITY

Allyl acetate and allyl alcohol are eye, dermal, and sensory irritants. Allyl acetate vapor is irritating to skin and mucous membranes and causes lacrimation and corneal burns. Contact with the liquid can cause first- to second-degree burns of the skin. Allyl alcohol is even more irritating; eye discomfort is experienced at 5 ppm and corneal necrosis and temporary blindness have been reported following exposure to 25 ppm (Arena and Drew, 1986). Both compounds are absorbed through intact skin, and enough allyl alcohol may be absorbed dermally to be toxic or lethal (Arena and Drew, 1986; HSDB 2001; NTP, unpublished data). In a study of the sensory irritant potential of allyl compounds in CF-1 mice, the concentration necessary to depress respiratory rates by 50% were 2.9, 3.9, and 2.9 ppm for allyl acetate, allyl alcohol, and acrolein, respectively (Nielsen *et al.*, 1984). No pulmonary irritation was observed at these concentrations. The acute toxicity of allyl alcohol is shown in Table 2.

TABLE 2
Acute Toxicity of Allyl Alcohol

Species	Route	LD_{50} or LC_{50}	Reference
Rat	Oral	64 mg/kg	Reid, 1972
Rat	Inhalation	165 ppm/4 hours	Belinsky *et al.*, 1985
Mouse	Oral	85 mg/kg	Badr, 1991
Mouse	Intraperitoneal	60 mg/kg	Belinsky *et al.*, 1985
Rabbit	Dermal	89 mg/kg	Silver and Murphy, 1978

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No information on the reproductive toxicity or teratogenic potential of either allyl acetate or allyl alcohol was found in the literature. Acrolein was evaluated for teratogenicity in New Zealand white rabbits and found negative at doses (0.1, 0.75, 2 mg/kg per day by gavage) that were not toxic to the pregnant females (Parent *et al.*, 1993).

CARCINOGENICITY

Neither allyl acetate nor allyl alcohol has been evaluated for carcinogenic potential. Allyl acetate is mutagenic in *Salmonella typhimurium* strains TA1535 and TA100 in the absence of activation but not in the presence of activation (Dean *et al.*, 1985). Allyl alcohol was mutagenic in V79 cells, but there are no reports of bacterial mutagenicity (Smith *et al.*, 1990).

Acrolein is a mutagen in bacteria and V79 cells and forms DNA adducts in human fibroblasts (Foiles *et al.*, 1989; Smith *et al.*, 1990; Wilson *et al.*, 1991). It is also a metabolite of the antineoplastic agent cyclophosphamide and is thought to be responsible for the hemorrhagic cystitis that occurs in patients treated with cyclophosphamide. Acrolein has also been implicated in the increased incidence of bladder cancer associated with cyclophosphamide therapy (Cannon *et al.*, 1991). Using immunoadsorbent and immunodot blot assays, acrolein adducts have been found in DNA from peripheral blood lymphocytes of cancer patients treated with cyclophosphamide. In several cases, these adducts have been shown to persist for long periods of time and be detectable in peripheral blood cells 5 years after the last cyclophosphamide treatment (McDiarmid *et al.*, 1991).

The carcinogenic potential of acrolein in animals is controversial. In an initiation/promotion protocol, 2 mg/kg acrolein was administered to male F344 rats by intraperitoneal injection twice weekly for 6 weeks (Cohen *et al.*, 1992). The animals were then fed a diet containing 3% uracil for 20 weeks followed by a control diet for 6 weeks. At the end of the 32-week study period, 60% of the male F344 rats that received acrolein and uracil had papillomas of the urinary bladder. Only 27% of males that received only uracil and none of the untreated controls had papillomas of the urinary bladder. This indicated that acrolein has initiating activity in the rat bladder. Because of toxicity associated with administration of 2 mg/kg acrolein for more than 6 weeks, the promoting activity could not be evaluated.

Increased incidences of adrenal cortical adenomas in F344 rats administered acrolein in drinking water at concentrations of 100, 250, or 625 ppm were reported by Lijinsky and Reuber (1987); however, the group sizes were small (20 animals), and no stability was reported for the aqueous acrolein solutions. In a more recent study, male and female Sprague-Dawley rats were given doses of 0.05, 0.5, or 2.4 mg/kg acrolein in water by gavage daily for 24 months (Parent *et al.*, 1992). Although survival of 2.4 mg/kg animals was reduced compared to vehicle controls, no chemical-related increases in the incidences of neoplastic or nonneoplastic lesions were observed in this study. The carcinogenic potential of acrolein has also been examined in CD-1 mice (Parent *et al.*, 1991). Groups of 70 male or female mice received doses of 0, 0.5, or 2.0 mg/kg acrolein in distilled water daily by gavage for 2 years, and additional groups of 75 males or females received 4.5 mg/kg. Survival and mean body weights of 4.5 mg/kg males were reduced, but survival and mean body weights of all groups of females were similar to those of the vehicle controls. Complete histopathology conducted on 4.5 mg/kg animals and vehicle controls revealed no carcinogenic response associated with acrolein administration.

GENETIC TOXICITY

Only one published mutagenicity data set for allyl acetate was identified. This was a review article containing test results but no data (Dean *et al.*, 1985). In this study, allyl acetate was positive in *S. typhimurium* strains TA100 and TA1535 without S9; no mutagenic activity was seen with S9, or in strains TA1537, TA1538, or TA98 with or without S9. In addition, allyl acetate was reported to be negative in the *in vitro* rat liver chromosomal aberrations test.

Allyl alcohol has not been tested extensively for mutagenicity. Negative results were reported for allyl alcohol in standard plate incorporation *Salmonella* gene mutation assays using a variety of tester strains (Bignami *et al.*, 1977; Rosen *et al.*, 1980); a modified liquid suspension (preincubation) assay with allyl alcohol (reported purity of 99.9%) gave positive results in TA100 in the absence of S9 activation and negative results with S9 (Lutz *et al.*, 1982). *In vivo*, allyl alcohol was tested for induction of dominant lethal mutations and other reproductive effects in male Sprague-Dawley rats administered 25 mg/kg allyl alcohol per day for up to 33 weeks; no dominant lethality, or increased preimplantation or postimplantation loss, was observed (Jenkinson and Anderson, 1990).

The mutagenicity of acrolein has been difficult to characterize due to its extreme electrophilicity, which causes it to react readily with a variety of nucleophilic compounds and produces marked toxicity (Beauchamp *et al.*, 1985). Conflicting results have been reported in several assays. Acrolein has demonstrated direct DNA interaction in studies designed to measure DNA adduct formation (Henschler and Eder, 1986; Foiles *et al.*, 1990; Eder *et al.*, 1993). It has been shown to be mutagenic in *S. typhimurium* strains TA100, TA104, and TA1535 (Hales, 1982; Lutz *et al.*, 1982; Haworth *et al.*, 1983; Marnett *et al.*, 1985; Parent *et al.*, 1996b). Positive results were reported in the absence of S9 in the cultured Chinese hamster ovary (CHO) cell test for induction of sister chromatid exchanges, but no increases in the frequencies of chromosomal aberrations were noted in CHO cells treated with acrolein, with or without S9 (Galloway *et al.*, 1987). Conflicting results have been reported for *in vitro* mammalian cell mutagenicity assays. A strong dose-related increase in 6-thioguanine resistant mutants was reported after treatment of fibroblast cultures derived from xeroderma pigmentosum (XP) patients, but not in normal human fibroblast cultures (Curren *et al.*, 1988); effective concentrations were less than 1.0 μM in XP cells. Negative results were reported in the CHO/HGPRT mutation assay with acrolein concentrations of 0.2 to 2 nM/mL in the absence of S9, and up to 8 nM/mL with rat S9. Negative results were reported in *Drosophila* sex-linked recessive lethal assays in larvae exposed to 800 ppm acrolein via feeding (Zimmering *et al.*, 1989) and in adults treated either by feeding (3,000 ppm) or by injection (200 ppm) (Zimmering *et al.*, 1985). However, administration of higher doses (3 to 7 mM) by injection into adult male *Drosophila* was reported to induce significant increases in sex-linked recessive lethal mutations but no effects in a test for sex-chromosome loss (Sierra *et al.*, 1991).

STUDY RATIONALE

Because of the high production volume and widespread use of these compounds, the potential for occupational and consumer exposure, and the lack of adequate toxicity and carcinogenicity data, allyl alcohol and allyl acetate were selected for in-depth studies. Allyl acetate and allyl alcohol are metabolized to acrolein, which is considerably more toxic than either parent compound. Therefore, a comparative toxicology study of allyl acetate, allyl alcohol, and acrolein was conducted in the same animal strains and at the same laboratory. If the toxic responses are similar for the three compounds, the major hazard posed by allyl acetate and allyl alcohol is likely the formation of acrolein. Because acrolein has been evaluated in 2-year studies (Parent *et al.*, 1991, 1992), evaluations beyond prechronic studies may not be necessary. If, however, the toxicities are substantially different, additional evaluations may be necessary.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Allyl acetate (lot 0425EF), allyl alcohol (lot 00501TF), and acrolein (lot 11163AG) were obtained from Aldrich Chemical Company (Milwaukee, WI). Information on identity and purity were provided by the manufacturer; identity of all chemicals and purity of acrolein were confirmed by the study laboratory, Battelle Columbus Laboratories (Columbus, OH) (Appendix H). Reports on analyses performed in support of the allyl acetate, allyl alcohol, and acrolein studies are on file at the National Institute of Environmental Health Sciences.

Allyl acetate and allyl alcohol, colorless liquids, and acrolein, a yellow liquid, were identified by infrared spectroscopy. Each spectrum was consistent with a literature reference (*Aldrich*, 1985) and with that expected for the structure. Gas chromatography data provided by the manufacturer indicated a purity of approximately 93.3% for allyl acetate, 98.8% for allyl alcohol, and 98.8% for acrolein. Titration data from the manufacturer indicated 7.74% water for acrolein. Gas chromatographic analyses performed by the study laboratory indicated no organic impurities. The combined data from the manufacturer and study laboratory indicated an overall purity of greater than 90% for acrolein.

Throughout the studies, the bulk chemicals were stored in glass bottles at approximately 5° C (allyl acetate and acrolein) or room temperature (allyl alcohol). Reanalyses by the study laboratory with high-performance liquid chromatography (allyl acetate) or gas chromatography (allyl alcohol and acrolein) indicated no degradation of the bulk chemicals.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing the chemical with 0.5% aqueous methylcellulose to form a suspension (allyl acetate) or solution (allyl alcohol and acrolein) (Table H1). Homogeneity and stability studies of the 0.8 and 20 mg/mL allyl acetate formulations and stability studies of 0.6 and 10 mg/mL allyl alcohol formulations and 0.125 and 2 mg/mL acrolein formulations were performed by the study laboratory. Allyl acetate formulations were analyzed with high-performance liquid chromatography. The stability of the allyl alcohol and acrolein formulations was analyzed with gas chromatography. Homogeneity was confirmed, and the stability of the allyl acetate and allyl alcohol dose formulations was confirmed for at least 21 days (allyl acetate) or 35 days (allyl alcohol) at room temperature or 5° C when stored sealed and protected from light. The stability of the acrolein formulations was confirmed for 7 days (0.125 mg/mL) or 14 days (2 mg/mL) at 5° C when stored sealed and protected from light.

Periodic analyses of the dose formulations were conducted by the study laboratory using high-performance liquid chromatography (allyl acetate) or gas chromatography (Tables H2, H3, and H4). Dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples were also analyzed at most time points. All allyl acetate formulations were within 10% of the target concentrations except one rat formulation; this formulation, which was 11% greater than the target concentration, was used for dosing. All but two animal room samples for rats and four for mice were more than 10% below the target concentrations. In the allyl alcohol studies, 11 of 15 dose formulations for rats and 10 of 15 for mice were within 10% of the target concentrations; all dose formulations that were not within specifications were remixed and were found to be within 10% of the target concentrations. Nine of 10 animal room samples of these dose formulations for rats and mice were within 10% of the target concentrations. All acrolein formulations for rats and mice were within 10% of the target concentrations; 7 of 15 animal room samples for rats and all but one for mice were more than 10% below the target concentrations. The chemical losses shown by the animal room sample analyses, particularly for the lower doses, were thought to be related to the volatility of the three chemicals.

TOXICITY STUDY DESIGNS

Core Studies

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, New York). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 11 or 12 (rats) or 14 or 15 (mice) days and were 6 (rats) or 7 (mice) weeks old on the first day of the studies. At the end of the quarantine period, 4 weeks after the study began, and at study termination, blood samples were collected from five male and five female rats and mice. 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 received allyl acetate, allyl alcohol, or acrolein in 0.5% methylcellulose (5 mL methylcellulose/kg body weight) by gavage at doses of 0, 6, 12, 25, 50, or 100 mg/kg body weight; 0, 1.5, 3, 6, 12, or 25 mg/kg; or 0, 0.75, 1.25, 2.5, 5, or 10 mg/kg; respectively, 5 days per week for 14 weeks. Groups of 10 male and 10 female mice received allyl acetate, allyl alcohol, or acrolein in 0.5% methylcellulose (10 mL methylcellulose/kg body weight) by gavage at doses of 0, 8, 16, 32, 62.5, or 125 mg/kg; 0, 3, 6, 12, 25, or 50 mg/kg; or 0, 1.25, 2.5, 5, 10, or 20 mg/kg; respectively, 5 days per week for 14 weeks. Vehicle controls received 0.5% aqueous methylcellulose at 5 mL/kg (rats) or 10 mL/kg (mice). Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings were recorded weekly for core study rats and mice. Core study animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 3.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all vehicle control animals, all animals in the highest dosed groups with at least 60% survivors at the time of scheduled sacrifice, all animals in higher dosed groups, and all animals that died before the end of the studies. Selected organs were examined in all lower dosed groups in all studies. Table 3 lists the tissues and organs routinely examined.

Upon completion of the laboratory pathologist's histopathologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

Supplemental Evaluations

Clinical Pathology

On days 4 and 23 of the studies, blood was collected from the retroorbital sinus of clinical pathology study rats anesthetized with a CO_2:O_2 mixture for hematology and clinical chemistry analyses. At the end of the 14-week studies, blood was collected from the retroorbital sinus of core study rats and mice for hematology analyses and from core study rats for clinical chemistry analyses. Blood for hematology analyses was placed in tubes with potassium-EDTA (Sarstedt, Inc., Nümbrecht, Germany), and blood for clinical chemistry analyses was placed in tubes without anticoagulant and allowed to clot, and the serum was separated by centrifugation. Hematology determinations were made with a Serono-Baker System 9000 hematology analyzer (Serono-Baker Diagnostics, Allentown, PA) with reagents obtained from the manufacturer. Differential leukocyte counts and morphologic evaluation of blood cells were determined by light microscopy from blood smears stained with modified Wright-Giemsa. Smears from blood samples stained with new methylene blue were examined microscopically for quantitative determination of reticulocytes. Clinical chemistry variables were measured with a Hitachi 704® chemistry analyzer (Boehringer Mannheim, Indianapolis, IN). Reagent for assays of sorbitol dehydrogenase was obtained from Sigma Chemical Company (St. Louis, MO); other reagents were obtained from the manufacturer. The hematology and clinical chemistry parameters evaluated are listed in Table 3.

3-Hydroxypropyl Mercapturic Acid Concentrations

Urine was collected from all core study rats and mice after the first gavage dose was administered and again after 45 doses. Approximately 15 to 30 minutes after dosing, animals were placed in metabolism cages for a 24-hour period. Rats were housed individually in metabolism cages during urine collection; mice receiving allyl acetate or acrolein and female mice receiving allyl alcohol were housed five animals per cage. Male mice receiving allyl alcohol were housed five animals per cage during the first urine collection and individually during the second urine collection. Feed and water were available *ad libitum*. Urine collection tubes were kept on ice during the entire collection period. The urine samples were kept frozen at approximately −20° C until they were shipped on dry ice to CEDRA Corporation (Austin, TX) for determination of 3-hydroxypropyl mercapturic acid concentrations. Urine samples were analyzed using a liquid chromatography-mass spectrometry method (CEDRA, 1996).

Sperm Motility and Vaginal Cytology Evaluations

At the end of the 14-week studies, samples were collected for sperm motility and vaginal cytology evaluations on core study rats administered 0, 12, 25, or 50 mg/kg allyl acetate or 0, 6, 12, or 25 mg/kg allyl alcohol and core study mice administered 0, 8 (males), 16, 32, or 62.5 (females) mg/kg allyl acetate or 0, 12, 25 or 50 mg/kg allyl alcohol. The parameters evaluated are listed in Table 3. Methods used were those described in the NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1991). 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 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% dimethylsulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

TABLE 3
Experimental Design and Materials and Methods in the 14-Week Gavage Studies of Allyl Acetate, Allyl Alcohol, and Acrolein

Allyl Acetate Studies	Allyl Alcohol Studies	Acrolein Studies
Study Laboratory Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$
Animal Source Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies Rats: 11 days (males) or 12 days (females) Mice: 14 days (males) or 15 days (females)	Same as allyl acetate studies	Same as allyl acetate studies
Average Age When Studies Began Rats: 6 weeks Mice: 7 weeks	Same as allyl acetate studies	Same as allyl acetate studies
Date of First Dose Rats: January 23, 1995 (males) or January 24, 1995 (females) Mice: January 26, 1995 (males) or January 27, 1995 (females)	Rats: February 20, 1995 (males) or February 21, 1995 (females) Mice: February 16, 1995 (males) or February 17, 1995 (females)	Rats: March 20, 1995 (males) or March 21, 1995 (females) Mice: March 16, 1995 (males) or March 17, 1995 (females)
Duration of Dosing 14 weeks (5 days/week)	Same as allyl acetate studies	Same as allyl acetate studies
Date of Last Dose Rats: April 24, 1995 (core study males) or April 25, 1995 (core study females) Mice: April 27, 1995 or April 28, 1995	Rats: May 22, 1995 (core study males) or May 23, 1995 (core study females) Mice: May 18, 1995 or May 19, 1995	Rats: June 19, 1995 (core study males) or June 20, 1995 (core study females) Mice: June 15, 1995 or June 16, 1995
Necropsy Dates Rats: April 24, 1995 (males) or April 25, 1995 (females) Mice: April 27, 1995 (males) or April 28, 1995 (females)	Rats: May 22, 1995 (males) or May 23, 1995 (females) Mice: May 18, 1995 (males) or May 19, 1995 (females)	Rats: June 19, 1995 (males) or June 20, 1995 (females) Mice: June 15, 1995 (males) or June 16, 1995 (females)
Average Age at Necropsy Rats: 19 weeks Mice: 20 weeks	Rats: 19 weeks Mice: 20 weeks	Rats: 19 weeks Mice: 20 weeks
Size of Study Groups 10 males and 10 females	Same as allyl acetate studies	Same as allyl acetate studies
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as allyl acetate studies	Same as allyl acetate studies

TABLE 3
Experimental Design and Materials and Methods in the 14-Week Gavage Studies of Allyl Acetate, Allyl Alcohol, and Acrolein

Allyl Acetate Studies	Allyl Alcohol Studies	Acrolein Studies
Animals per Cage Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 5 Mice: 1 (males) or 5 (females)
Method of Animal Identification Tail tattoo	Tail tattoo	Tail tattoo
Diet NTP-2000 pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as allyl acetate studies	Same as allyl acetate studies
Water Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as allyl acetate studies	Same as allyl acetate studies
Cages Polycarbonate (Lab Products, Inc., Maywood, NJ), changed twice weekly (rats and female mice) or weekly (male mice)	Same as allyl acetate studies	Same as allyl acetate studies
Bedding Sani-Chip® hardwood chips (P.J. Murphy Forest Products, Corp., Montville, NJ), changed twice weekly (rats and female mice) or weekly (male mice)	Same as allyl acetate studies	Same as allyl acetate studies
Cage Filters Spun-bonded DuPont 2024 polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as allyl acetate studies	Same as allyl acetate studies
Racks Stainless steel (Lab Products, Inc., Maywood, NJ), changed every 2 weeks	Same as allyl acetate studies	Same as allyl acetate studies
Animal Room Environment Temperature: 70° ± 3° F Relative humidity: 50%-15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Same as allyl acetate studies	Same as allyl acetate studies
Doses Rats: 0, 6, 12, 25, 50, or 100 mg/kg in 0.5% methylcellulose by gavage (dosing volume 5 mL/kg) Mice: 0, 8, 16, 32, 62.5, or 125 mg/kg in 0.5% methylcellulose by gavage (dosing volume 10 mL/kg)	Rats: 0, 1.5, 3, 6, 12, or 25 mg/kg in 0.5% methylcellulose by gavage (dosing volume 5 mL/kg) Mice: 0, 3, 6, 12, 25, or 50 mg/kg in 0.5% methylcellulose by gavage (dosing volume 10 mL/kg)	Rats: 0, 0.75, 1.25, 2.5, 5, or 10 mg/kg in 0.5% methylcellulose by gavage (dosing volume 5 mL/kg) Mice: 0, 1.25, 2.5, 5, 10, or 20 mg/kg in 0.5% methylcellulose by gavage (dosing volume 10 mL/kg)

TABLE 3
Experimental Design and Materials and Methods in the 14-Week Gavage Studies of Allyl Acetate, Allyl Alcohol, and Acrolein

Allyl Acetate Studies	Allyl Alcohol Studies	Acrolein Studies
Type and Frequency of Observation		
Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly.	Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly.	Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly.
Method of Sacrifice		
CO_2 asphyxiation	Same as allyl acetate studies	Same as allyl acetate studies
Necropsy		
Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.
Clinical Pathology		
Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 23 for hematology and clinical chemistry analyses. Blood was collected from the retroorbital sinus of core study rats and mice at the end of the studies for hematology analyses and core study rats for clinical chemistry analyses. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and total leukocyte counts and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, total bile acids, and creatine kinase	Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 23 for hematology and clinical chemistry analyses. Blood was collected from the retroorbital sinus of core study rats and mice at the end of the studies for hematology analyses and core study rats for clinical chemistry analyses. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and total leukocyte counts and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, total bile acids, and creatine kinase	Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 23 for hematology and clinical chemistry analyses. Blood was collected from the retroorbital sinus of core study rats and mice at the end of the studies for hematology analyses and core study rats for clinical chemistry analyses. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and total leukocyte counts and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, total bile acids, and creatine kinase

TABLE 3
Experimental Design and Materials and Methods in the 14-Week Gavage Studies of Allyl Acetate, Allyl Alcohol, and Acrolein

Allyl Acetate Studies	Allyl Alcohol Studies	Acrolein Studies
Histopathology Complete histopathology was performed on all vehicle control animals, all animals in the highest dosed groups with at least 60% survivors at the time of scheduled sacrifice, all animals in higher dosed groups, and all animals that died before the end of the studies. Selected organs were examined in all lower dosed groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, brain, clitoral gland, esophagus, eye, femur, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (with adjacent skin), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spinal cord/sciatic nerve/muscle, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). Organs examined in the lower dosed groups included the liver and forestomach of rats and mice and the glandular stomach of mice.	Complete histopathology was performed on all vehicle control animals, all animals in the highest dosed groups with at least 60% survivors at the time of scheduled sacrifice, all animals in higher dosed groups, and all animals that died before the end of the studies. Selected organs were examined in all lower dosed groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, brain, clitoral gland, esophagus, eye, femur, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (with adjacent skin), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spinal cord/sciatic nerve/muscle, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). Organs examined in the lower dosed groups included the liver and forestomach of rats and mice and the glandular stomach of mice.	Complete histopathology was performed on all vehicle control animals, all animals in the highest dosed groups with at least 60% survivors at the time of scheduled sacrifice, all animals in higher dosed groups, and all animals that died before the end of the studies. Selected organs were examined in all lower dosed groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, brain, clitoral gland, esophagus, eye, femur, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (with adjacent skin), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spinal cord/sciatic nerve/muscle, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. Organs examined in the lower dosed groups included the liver, forestomach, and glandular stomach of rats and mice.

TABLE 3
Experimental Design and Materials and Methods in the 14-Week Gavage Studies of Allyl Acetate, Allyl Alcohol, and Acrolein

Allyl Acetate Studies	Allyl Alcohol Studies	Acrolein Studies
Sperm Motility and Vaginal Cytology Evaluations		
At the end of the studies, sperm samples were collected from all core study male rats administered 0, 12, 25, or 50 mg/kg allyl acetate and all core study male mice administered 0, 8, 16, or 32 mg/kg allyl acetate for sperm motility evaluations. The following parameters were evaluated: sperm concentration, motility, and spermatid head count. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from all core study female rats administered 0, 12, 25, or 50 mg/kg allyl acetate, and all core study female mice administered 0, 16, 32, or 62.5 mg/kg allyl acetate for vaginal cytology evaluations. The following parameters were evaluated: the relative frequency of estrous stages and estrous cycle length.	At the end of the studies, sperm samples were collected from all core study male rats administered 0, 6, 12, or 25 mg/kg allyl alcohol and all core study male mice administered 0, 12, 25 or 50 mg/kg allyl alcohol for sperm motility evaluations. The following parameters were evaluated: sperm concentration, motility, and spermatid head count. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from all core study female rats administered 0, 6, 12, or 25 mg/kg allyl alcohol and all core study female mice administered 0, 12, 25 or 50 mg/kg allyl alcohol for vaginal cytology evaluations. The following parameters were evaluated: the relative frequency of estrous stages and estrous cycle length.	None
3-Hydroxypropyl Mercapturic Acid Concentrations		
Urine was collected from all core study rats and mice after administration of the first gavage dose and after administration of the 45th gavage dose. The urine samples were analyzed for 3-hydroxypropyl mercapturic acid.	Same as allyl acetate studies	Same as allyl acetate studies

STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

The incidences of lesions as presented in Appendixes A and B are given 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, a procedure based on the overall proportion of affected animals, was used to determine significance (Gart *et al.*, 1979).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and vehicle control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, 3-hydroxypropyl mercapturic acid concentration, 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 dose levels.

QUALITY ASSURANCE

The 14-week studies of allyl acetate, allyl alcohol, and acrolein were conducted in compliance with Food and Drug Administration Good Laboratory Practices Regulations (21 CFR, Part 58). The Quality Assurance Unit of Battelle Columbus Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the study.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least four doses of the test chemical. The high dose was limited by toxicity in all the *Salmonella typhimurium* tests, except in the allyl acetate study performed at Environmental Health Research and Testing, Inc., where, in the absence of toxicity, 10,000 μg/plate was selected as the high dose. All positive trials were repeated under the conditions which elicited the positive response.

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.

Allyl acetate and allyl alcohol: Testing was performed as reported by Mortelmans *et al.* (1986). Allyl acetate and allyl alcohol were sent to the laboratories as a coded aliquot from Radian Corporation (Austin, TX). Each chemical was incubated with the *S. typhimurium* tester strains TA97, TA98, TA100, 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.

Acrolein: Acrolein was tested at two independent laboratories. At the first laboratory, testing was performed with a preincubation protocol as reported by Haworth *et al.* (1983). Acrolein was sent to the laboratory as a coded aliquot from Radian Corporation. It was incubated with the *S. typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

At the second laboratory, acrolein was tested as a vapor by a desiccator procedure (Zeiger *et al.*, 1992). Acrolein was sent to the laboratory as a coded aliquot from Radian Corporation. The *S. typhimurium* strains TA97, TA98, TA100, and TA1535 and S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) or buffer were incorporated into top agar supplemented with L-histidine and d-biotin and poured onto the surfaces of minimal glucose agar plates. The lids of the plates were removed and the plates were stacked on a perforated porcelain plate in a 9-liter desiccator jar containing a magnetic stirring bar. A measured volume

of acrolein, in liquid form, was introduced into a glass petri dish suspended below the porcelain plate. The dose was expressed as μL acrolein per desiccator (chamber). The desiccator was then sealed and placed on a magnetic stirrer in a 37° C incubator. After 24 hours, the plates were removed from the desiccator and incubated in air at 37° C for an additional 24 hours. Upon completion of the incubation period, histidine-independent mutant colonies were counted.

Acrolein was also tested at the second laboratory by the standard preincubation procedure in strains TA1538 and TA98, with and without S9 (TA98), in a procedure similar to that described for acrolein (Zeiger *et al.*, 1992).

Chinese Hamster Ovary Cell Cytogenetics Protocols

Testing was performed as reported by Galloway *et al.* (1987). Acrolein was supplied as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent vehicle and positive controls and of three doses of acrolein; the high dose was limited by toxicity. A single flask per dose was used.

Sister Chromatid Exchange Test

In the SCE test without S9, CHO cells were incubated for 26 hours with acrolein in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing acrolein was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with acrolein, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no acrolein. Incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.005$), in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test

In the Abs test without S9, cells were incubated in McCoy's 5A medium with acrolein for 12 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with acrolein and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 12 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test.

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

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

Drosophila melanogaster Test Protocol

The assays for induction of sex-linked recessive lethal (SLRL) mutations were performed with adult flies as described by Zimmering *et al.* (1985) and with larvae as described by Zimmering *et al.* (1989). Acrolein was supplied as a coded aliquot from Radian Corporation. Acrolein was assayed in the SLRL test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no response was obtained, acrolein was retested by injection into adult males.

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

Toxicity tests were performed to set concentrations of acrolein at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. Canton-S males were allowed to feed for 72 hours on a solution of acrolein in 5% sucrose. In the injection experiments, 24 to 72-hour old Canton-S males were treated with a solution of acrolein dissolved in saline and allowed to recover for 24 hours. A concurrent saline control group was also included. In the adult exposures, treated males were mated to three *Basc* females for 3 days and were given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings was treated at successively earlier postmeiotic stages). For the larval feeding experiment, Canton-S males and females were mated and eggs were exposed in vials with standard cornmeal feed containing acrolein in solvent (distilled water) or solvent alone (Zimmering *et al.*, 1989). Adult emergent males were mated at approximately 24 hours of age with two successive harems of three to five *Basc* females to establish two single-day broods. For both the adult and larval exposure experiments, F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

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

Rat Bone Marrow Micronucleus Test Protocol

Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by the chemical exposure. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male F344/N rats were dosed by gavage (allyl acetate) or injected intraperitoneally (allyl alcohol) three times at 24-hour intervals, with the test chemical dissolved in corn oil (allyl acetate) or phosphate-buffered saline (allyl alcohol). Vehicle control animals were administered vehicle only. The positive control animals received injections of cyclophosphamide. The animals were killed 24 hours after the third dose, and blood smears were prepared from the bone marrow cells obtained from the femurs. Air-dried smears were fixed

and stained with acridine orange; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of five animals per dose group.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the vehicle control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single 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.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 14-week toxicity studies, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of 10 animals per dose group. In addition, the percentage of PCEs among the total erythrocyte population in the peripheral blood was scored for each dose group as a measure of toxicity.

The results were tabulated as described for PCEs in the bone marrow micronucleus test. Results of the 14-week studies were accepted without repeat tests, because additional test data could not be obtained.

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

Allyl acetate: All male and female rats in the 100 mg/kg groups died or were killed moribund by day 8; there were no other deaths (Table 4). Final mean body weights and mean body weight gains of male rats administered 12 or 50 mg/kg and mean body weight gains of 6 mg/kg males were significantly less than those of the vehicle controls. The final mean body weights and mean body weight gains of female rats were similar to those of the vehicle control group (Table 4 and Figure 2). Clinical findings included pallor and eye or nasal discharge in males and females and ruffled fur, lethargy, diarrhea, and thinness in males in the 100 mg/kg groups.

Allyl alcohol: All rats survived to the end of the study except one female rat in the 6 mg/kg group that was removed from the study on day 57 in a moribund condition (Table 5). The final mean body weights and mean body weight gains of male and female rats were similar to those of the vehicle controls (Table 5 and Figure 3). No clinical findings were observed in dosed male or female rats.

Acrolein: Eight males and eight females in the 10 mg/kg groups died by week 9 of the study (Table 6). Two males in the 2.5 and 5 mg/kg groups and one or two females in the 1.25, 2.5, and 5 mg/kg groups also died early; two of these deaths were gavage accidents. Final mean body weights and mean body weight gains of male and female rats in the 10 mg/kg groups were significantly less than those of the vehicle controls (Table 6 and Figure 4). Clinical findings included abnormal breathing, eye or nasal discharge, ruffled fur, and thinness in males and females in the 10 mg/kg groups; two females in this group were also lethargic.

The concentrations of 3-hydroxy mercapturic acid in the urine of rats after one or 45 doses of allyl acetate or allyl alcohol increased linearly with dose (Tables F1 and F2). In rats dosed with acrolein, the concentrations increased nonlinearly with dose at the first time point and linearly with dose at the second time point (except at 10 mg/kg) (Table F3).

TABLE 4
Survival and Body Weights of Rats in the 14-Week Gavage Study of Allyl Acetate

Dose (mg/kg)	Survival[a]	Mean Body Weight[b]			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	114 ± 4	340 ± 5	226 ± 4	
6	10/10	115 ± 4	326 ± 6	211 ± 5*	96
12	10/10	116 ± 4	317 ± 5*	201 ± 3**	93
25	10/10	117 ± 4	331 ± 4	214 ± 4	97
50	10/10	110 ± 5	319 ± 7*	209 ± 3**	94
100	0/10[c]	116 ± 3	—	—	—
Female					
0	10/10	103 ± 2	193 ± 3	89 ± 2	
6	10/10	104 ± 3	186 ± 2	82 ± 3	96
12	10/10	104 ± 2	191 ± 4	86 ± 3	99
25	10/10	103 ± 2	190 ± 3	87 ± 2	99
50	10/10	100 ± 2	187 ± 4	86 ± 2	97
100	0/10[d]	105 ± 2	—	—	—

* 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 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] Week of death: 1, 1, 1, 1, 1, 1, 1, 1, 2, 2
[d] Week of death: 1

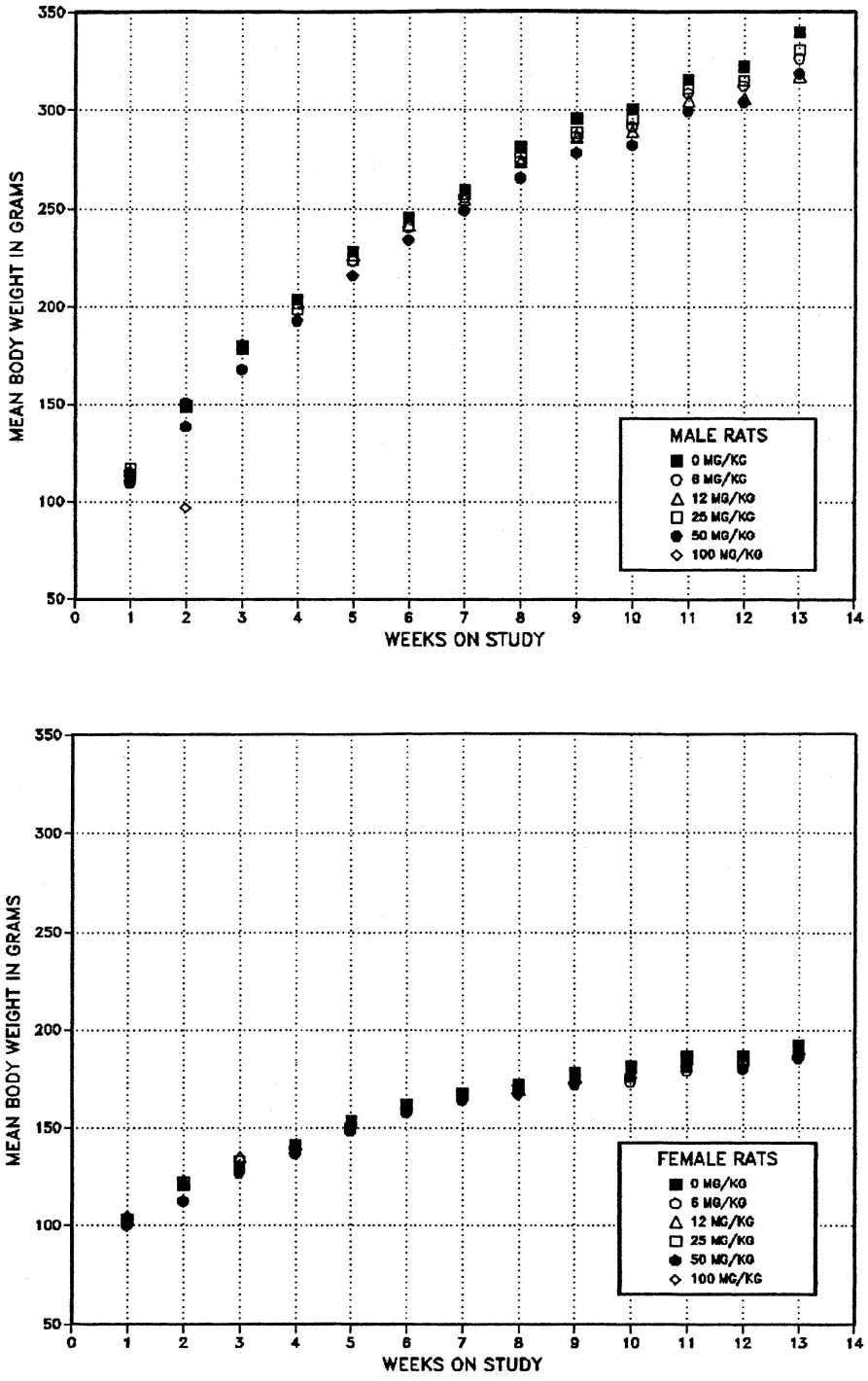


FIGURE 2
Body Weights of Rats Administered Allyl Acetate by Gavage for 14 Weeks

TABLE 5
Survival and Body Weights of Rats in the 14-Week Gavage Study of Allyl Alcohol

Dose (mg/kg)	Survival[a]	Mean Body Weight[b]			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	112 ± 3	338 ± 6	226 ± 4	
1.5	10/10	113 ± 3	331 ± 5	218 ± 5	98
3	10/10	112 ± 3	322 ± 5	210 ± 6	95
6	10/10	112 ± 3	335 ± 6	223 ± 7	99
12	10/10	112 ± 3	337 ± 6	225 ± 4	100
25	10/10	111 ± 3	334 ± 7	223 ± 5	99
Female					
0	10/10	105 ± 3	201 ± 4	96 ± 2	
1.5	10/10	105 ± 3	196 ± 3	91 ± 2	97
3	10/10	104 ± 3	195 ± 5	92 ± 3	97
6	9/10[c]	104 ± 3	204 ± 4	100 ± 3	101
12	10/10	105 ± 3	203 ± 4	98 ± 4	101
25	10/10	106 ± 2	204 ± 3	98 ± 3	101

[a] Number of animals surviving at 14 weeks/number inititally 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] One female was removed from the study during week 9. The body weights of this animal were not included in the means.

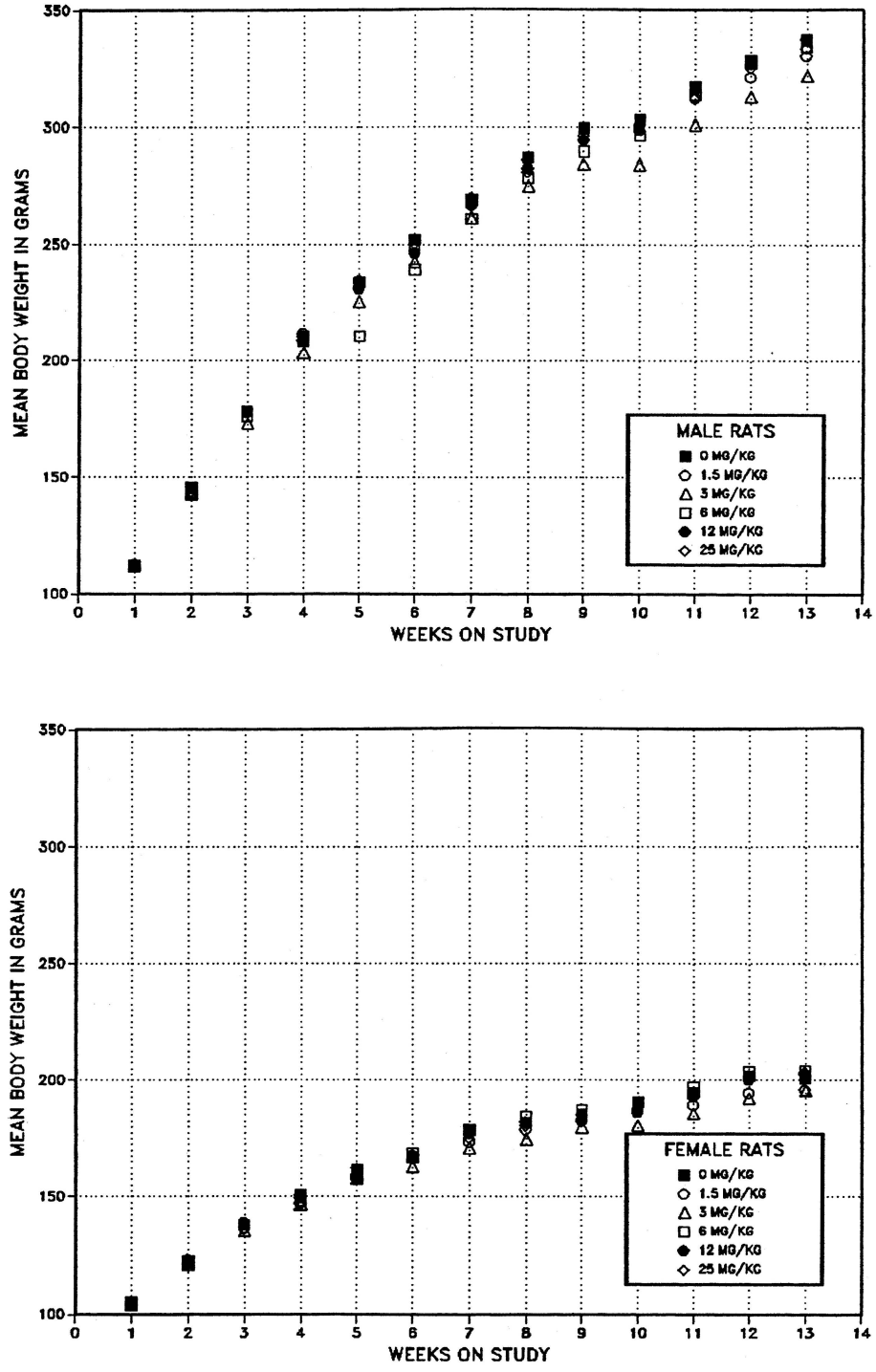


FIGURE 3
Body Weights of Rats Administered Allyl Alcohol by Gavage for 14 Weeks

TABLE 6
Survival and Body Weights of Rats in the 14-Week Gavage Study of Acrolein

Dose (mg/kg)	Survival[a]	Mean Body Weight[b]			Final Weight Relative to controls (%)
		Initial	Final	Change	
Male					
0	10/10	116 ± 4	337 ± 6	222 ± 6	
0.75	10/10	117 ± 3	336 ± 4	219 ± 6	100
1.25	10/10	117 ± 3	336 ± 6	219 ± 5	100
2.5	8/10[c]	118 ± 4	348 ± 6	229 ± 3	103
5	8/10[c]	118 ± 3	331 ± 8	212 ± 6	98
10	2/10[d]	118 ± 4	263 ± 10**	161 ± 6**	78
Female					
0	10/10	106 ± 2	195 ± 4	89 ± 4	
0.75	10/10	105 ± 3	196 ± 2	91 ± 2	101
1.25	9/10[e]	108 ± 2	198 ± 3	89 ± 3	101
2.5	8/10[f]	106 ± 2	190 ± 3	86 ± 3	97
5	9/10[g]	105 ± 2	188 ± 3	84 ± 3	97
10	2/10[h]	105 ± 3	176 ± 7*	65 ± 2**	90

* 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 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 Week of death: 6, 7
d Week of death: 1, 2, 2, 2, 4, 6, 6, 7
e Week of death: 5
f Week of death: 3, 6
g Week of death: 7
h Week of death: 1, 3, 4, 4, 4, 6, 7, 9

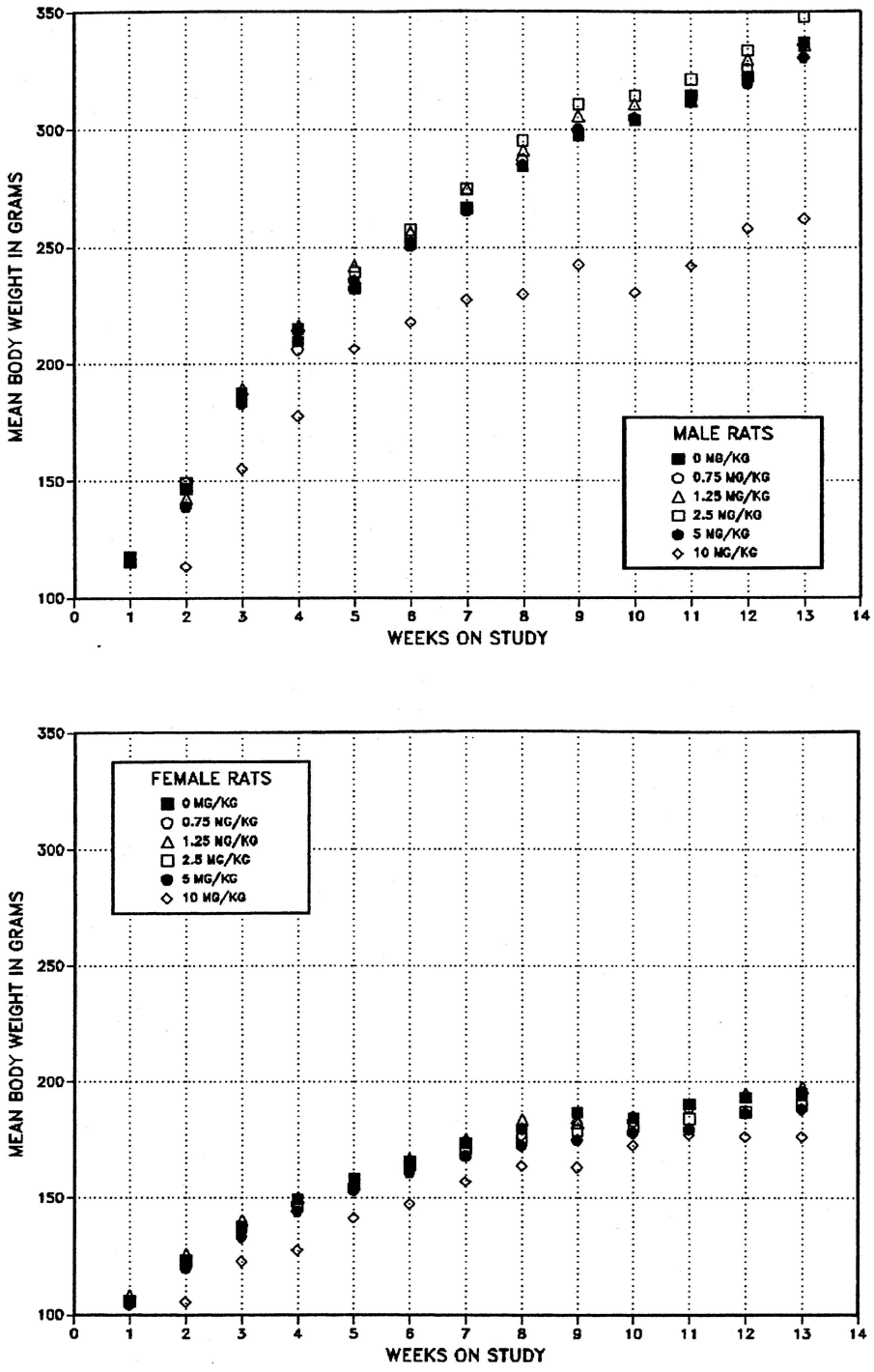


FIGURE 4
Body Weights of Rats Administered Acrolein by Gavage for 14 Weeks

Allyl acetate: The hematology and clinical chemistry data for rats are presented in Table C1, and selected data are presented in Table 7. On day 4, hematocrit values, hemoglobin concentrations, and erythrocyte counts were decreased in surviving 100 mg/kg males and females, and the decreases are consistent with a mild to marked anemia. The decrease in the erythron was accompanied by increases in reticulocyte and nucleated erythrocyte counts, suggesting a developing hematopoietic response to the anemia. Mean cell volume and mean cell hemoglobin in 100 mg/kg females were increased, and these increases are consistent with the increased numbers of circulating immature erythrocytes on day 4; also, mean cell hemoglobin concentration was increased, suggesting that the anemia might have been, in part, related to a hemolytic process. Platelet counts were markedly reduced in 100 mg/kg males (by approximately 77%) and females (by approximately 91%), suggesting that an acute loss or consumption of the circulating platelet mass occurred, such as with a disseminated intravascular coagulation. There was also a mild platelet count decrease in 50 mg/kg females; 50 mg/kg males were unaffected. Neutrophilia, evidenced by increased segmented neutrophil counts, occurred in 50 and 100 mg/kg males and females and is consistent with necrosis and inflammation observed microscopically in the forestomach and/or liver of rats in these groups. On day 4, there was evidence of a hepatocellular effect as demonstrated by increases in serum alanine aminotransferase and sorbitol dehydrogenase activities and bile acid concentrations in 50 (approximately 1.5- to 4-fold increase) and 100 mg/kg (ninefold or greater increase) males and females; a hepatocellular effect would be consistent with the liver lesions observed microscopically. Albumin and, consequently, total protein concentrations were significantly decreased and urea nitrogen concentrations were increased in 100 mg/kg males and females. Because no 100 mg/kg animals survived beyond day 8 of the study and because protein and urea concentrations can be influenced by liver function and nutritional/hydration status, the alterations in these variables were suspected to be related to the acute liver and/or forestomach toxicity in the 100 mg/kg rats.

On day 23 and/or at week 14, there were minimal decreases in mean cell volumes and mean cell hemoglobin values and increases in platelet counts in 50 mg/kg males and females; the mean cell hemoglobin concentration was minimally decreased at each time point in these females. The mechanism for the increased platelet counts is unknown. However, the changes in mean cell volumes, mean cell hemoglobin values, and mean cell hemoglobin concentrations suggest a change in hematopoiesis possibly through altered iron metabolism related to the inflammatory changes observed in the liver. The biochemical evidence of a liver effect that occurred in 50 mg/kg males and females on day 4 was observed on day 23 and, to a lesser extent, at week 14. The increases in segmented neutrophil counts that occurred in the 50 mg/kg males and females on day 4 ameliorated with time, and by week 14 occurred only in males. There were sporadic increases and decreases in various parameters at various time points that, in general, did not demonstrate a treatment relationship and/or were inconsistent between sexes; they were not considered toxicologically relevant.

The absolute and relative liver weights of 50 mg/kg females were significantly greater than those of the vehicle controls (Table D1).

TABLE 7
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	10	9	2
Day 23	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Hematocrit (%)						
Day 4	42.4 ± 0.7	41.9 ± 0.5	41.8 ± 0.4	40.7 ± 0.4	41.2 ± 0.6	35.7 ± 3.8
Day 23	46.1 ± 0.4	46.1 ± 0.5	45.9 ± 0.4	46.2 ± 0.5	44.5 ± 0.4	
Week 14	46.2 ± 0.6	46.4 ± 0.4	46.1 ± 0.4	45.9 ± 0.4	45.7 ± 0.5	
Hemoglobin (g/dL)						
Day 4	13.7 ± 0.2	13.5 ± 0.1	13.6 ± 0.2	13.2 ± 0.1	13.4 ± 0.2	11.9 ± 1.3
Day 23	15.3 ± 0.2	15.4 ± 0.1	15.2 ± 0.1	15.2 ± 0.2	14.9 ± 0.2	
Week 14	15.0 ± 0.2	15.2 ± 0.1	15.1 ± 0.1	15.0 ± 0.1	14.9 ± 0.1	
Erythrocytes (10^6/μL)						
Day 4	7.04 ± 0.14	7.04 ± 0.09	7.04 ± 0.08	6.81 ± 0.06	7.03 ± 0.10	6.16 ± 0.56
Day 23	7.61 ± 0.09	7.67 ± 0.10	7.65 ± 0.06	7.66 ± 0.09	7.58 ± 0.09	
Week 14	8.50 ± 0.13	8.62 ± 0.07	8.58 ± 0.07	8.51 ± 0.06	8.71 ± 0.09	
Mean cell volume (fL)						
Day 4	60.4 ± 0.5	59.5 ± 0.2	59.5 ± 0.3	59.6 ± 0.5	58.8 ± 0.1**	58.0 ± 1.0*
Day 23	60.6 ± 0.5	60.2 ± 0.3	60.1 ± 0.3	60.4 ± 0.4	58.9 ± 0.5	
Week 14	54.4 ± 0.3	53.7 ± 0.2	53.9 ± 0.1	54.0 ± 0.2	52.5 ± 0.2**	
Mean cell hemoglobin (pg)						
Day 4	19.5 ± 0.1	19.2 ± 0.1	19.4 ± 0.1	19.3 ± 0.2	19.1 ± 0.1	19.3 ± 0.4
Day 23	20.2 ± 0.1	20.1 ± 0.2	19.8 ± 0.1*	19.9 ± 0.1	19.6 ± 0.1**	
Week 14	17.7 ± 0.1	17.6 ± 0.1	17.6 ± 0.1	17.6 ± 0.1	17.1 ± 0.1**	
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.3 ± 0.2	32.3 ± 0.2	32.6 ± 0.1	32.4 ± 0.4	32.5 ± 0.1	33.4 ± 0.2
Day 23	33.2 ± 0.1	33.4 ± 0.2	33.1 ± 0.2	33.0 ± 0.2	33.4 ± 0.3	
Week 14	32.5 ± 0.1	32.7 ± 0.1	32.7 ± 0.1	32.7 ± 0.2	32.6 ± 0.1	
Platelets (10^3)						
Day 4	777.5 ± 34.5	905.7 ± 20.5*	893.3 ± 33.7*	863.2 ± 32.4	726.9 ± 49.1	179.5 ± 99.5
Day 23	779.3 ± 18.3	752.6 ± 12.0	742.4 ± 16.0	721.3 ± 17.0	831.9 ± 26.6	
Week 14	641.3 ± 10.1	650.4 ± 14.1	663.7 ± 10.9	696.0 ± 14.7*	804.9 ± 23.6**	
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	2
Day 23	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Total protein (g/dL)						
Day 4	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.4 ± 0.1	5.5 ± 0.1	4.5 ± 0.5*
Day 23	6.0 ± 0.1	6.1 ± 0.1	5.9 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	
Week 14	6.5 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.5 ± 0.0	6.6 ± 0.1	
Albumin (g/dL)						
Day 4	4.3 ± 0.1	4.2 ± 0.0	4.2 ± 0.1	4.1 ± 0.1*	3.8 ± 0.1**	2.8 ± 0.5**
Day 23	4.5 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	
Week 14	4.8 ± 0.1	4.6 ± 0.0	4.7 ± 0.0	4.7 ± 0.0	4.5 ± 0.1**	

TABLE 7
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	2
Day 23	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Alanine aminotransferase (IU/L)						
Day 4	68 ± 2	70 ± 2	68 ± 2	73 ± 3	99 ± 13	625 ± 205**
Day 23	64 ± 1	66 ± 2	65 ± 2	61 ± 2	141 ± 31*	
Week 14	88 ± 3	83 ± 3	95 ± 4	99 ± 5	112 ± 16	
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 2	14 ± 1	15 ± 1	15 ± 1	31 ± 6	217 ± 117
Day 23	24 ± 1	25 ± 2	22 ± 1	25 ± 2	53 ± 13**	
Week 14	29 ± 1	25 ± 2	28 ± 2	35 ± 3	39 ± 6	
Bile acids (μmol/L)						
Day 4	25.8 ± 2.6	20.9 ± 1.6	35.4 ± 2.5	30.9 ± 2.6	39.8 ± 6.3*	307.5 ± 25.5*
Day 23	15.0 ± 1.6	15.7 ± 1.0	17.0 ± 1.5	20.7 ± 2.2*	45.8 ± 8.3**	
Week 14	18.0 ± 1.0	21.8 ± 2.0	19.8 ± 2.7	21.2 ± 2.2	23.4 ± 2.3	
Female						
Hematology						
n						
Day 4	10	9	9	10	10	4
Day 23	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Hematocrit (%)						
Day 4	44.3 ± 0.5	45.7 ± 0.8	43.8 ± 0.7	43.5 ± 0.6	44.3 ± 0.6	25.2 ± 4.1*
Day 23	46.0 ± 0.5	46.1 ± 0.3	45.6 ± 0.5	46.2 ± 0.3	44.7 ± 0.5	
Week 14	44.5 ± 0.4	44.6 ± 0.6	43.9 ± 0.5	45.2 ± 0.4	43.0 ± 0.5	
Hemoglobin (g/dL)						
Day 4	14.2 ± 0.2	14.3 ± 0.3	13.8 ± 0.2	13.9 ± 0.2	14.1 ± 0.2	8.8 ± 1.3**
Day 23	15.2 ± 0.2	15.1 ± 0.1	15.0 ± 0.2	15.0 ± 0.1	14.6 ± 0.1**	
Week 14	14.7 ± 0.2	14.6 ± 0.2	14.5 ± 0.1	14.7 ± 0.1	14.0 ± 0.2**	
Erythrocytes (10^6/μL)						
Day 4	7.26 ± 0.09	7.39 ± 0.13	7.16 ± 0.11	7.06 ± 0.08	7.27 ± 0.08	3.84 ± 0.69*
Day 23	7.43 ± 0.08	7.45 ± 0.07	7.31 ± 0.09	7.39 ± 0.05	7.45 ± 0.09	
Week 14	7.63 ± 0.06	7.66 ± 0.10	7.52 ± 0.08	7.77 ± 0.08	7.69 ± 0.08	
Mean cell volume (fL)						
Day 4	61.2 ± 0.3	61.8 ± 0.3	61.1 ± 0.2	61.7 ± 0.2	61.0 ± 0.4	66.0 ± 1.4**
Day 23	62.1 ± 0.3	62.1 ± 0.2	62.4 ± 0.2	62.6 ± 0.3	60.1 ± 0.4**	
Week 14	58.4 ± 0.2	58.3 ± 0.2	58.3 ± 0.2	58.2 ± 0.2	55.9 ± 0.2**	
Mean cell hemoglobin (pg)						
Day 4	19.5 ± 0.1	19.3 ± 0.1	19.3 ± 0.2	19.7 ± 0.2	19.3 ± 0.1	23.5 ± 0.9*
Day 23	20.5 ± 0.2	20.3 ± 0.1	20.5 ± 0.1	20.3 ± 0.1	19.6 ± 0.2**	
Week 14	19.3 ± 0.2	19.1 ± 0.1	19.3 ± 0.2	18.9 ± 0.1*	18.1 ± 0.1**	

TABLE 7
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	9	9	10	10	4
Day 23	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.0 ± 0.2	31.3 ± 0.2	31.6 ± 0.3	31.9 ± 0.3	31.7 ± 0.2	35.4 ± 0.7
Day 23	33.1 ± 0.3	32.8 ± 0.2	32.9 ± 0.1	32.5 ± 0.1	32.7 ± 0.3	
Week 14	33.1 ± 0.3	32.8 ± 0.2	33.0 ± 0.3	32.4 ± 0.1	32.4 ± 0.1	
Platelets (10^3/μL)						
Day 4	954.9 ± 15.4	920.3 ± 31.2	941.7 ± 32.3	952.6 ± 22.3	711.3 ± 47.7**	82.3 ± 14.6**
Day 23	702.1 ± 18.6	713.6 ± 15.6	748.2 ± 13.9	731.4 ± 13.4	975.0 ± 30.3**	
Week 14	675.5 ± 9.6	662.4 ± 10.7	664.6 ± 6.5	686.8 ± 13.1	809.2 ± 40.6*	
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	4
Day 23	10	10	10	9	10	0
Week 14	10	10	10	10	10	0
Total Protein (g/dL)						
Day 4	5.6 ± 0.2	5.9 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	4.4 ± 0.2**
Day 23	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	5.9 ± 0.0	6.0 ± 0.1	
Week 14	6.7 ± 0.1	6.6 ± 0.1	6.4 ± 0.1*	6.4 ± 0.1*	6.3 ± 0.1**	
Albumin (g/dL)						
Day 4	4.5 ± 0.0	4.6 ± 0.1	4.3 ± 0.1*	4.3 ± 0.1	3.8 ± 0.1**	2.8 ± 0.2**
Day 23	4.6 ± 0.0	4.6 ± 0.1	4.5 ± 0.0	4.4 ± 0.0*	4.2 ± 0.0**	
Week 14	5.1 ± 0.1	5.0 ± 0.1	4.8 ± 0.1*	4.8 ± 0.1**	4.4 ± 0.0**	
Alanine aminotransferase (IU/L)						
Day 4	60 ± 2	51 ± 2	58 ± 2	54 ± 1	189 ± 34*	980 ± 2,251*
Day 23	53 ± 1	51 ± 1	53 ± 2	53 ± 2	61 ± 2	
Week 14	71 ± 3	75 ± 6	75 ± 5	69 ± 4	95 ± 10	
Sorbitol dehydrogenase (IU/L)						
Day 4	17 ± 1	12 ± 1	14 ± 1	15 ± 1	72 ± 19*	588 ± 366*
Day 23	20 ± 1	21 ± 1	23 ± 1	20 ± 1	24 ± 3	
Week 14	18 ± 1	22 ± 2	21 ± 2	20 ± 2	42 ± 7**	
Bile acids (μmol/L)						
Day 4	24.9 ±3.9	18.8 ± 1.8	19.6 ± 1.8	20.4 ± 1.2	100.2 ± 15.6**	256.3 ± 82.5**
Day 23	16.8 ± 1.9	17.6 ± 3.0	20.4 ± 1.9	21.0 ± 2.4	43.9 ± 4.7**	
Week 14	23.3 ± 2.2	26.6 ± 2.7	25.5 ± 4.2	27.1 ± 1.8	81.0 ± 12.6**	

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data were available for 100 mg/kg males or females on day 23 or at week 14 due to 100% mortality.

No differences were found in sperm motility or vaginal cytology parameters between dosed and vehicle control rats (Tables E1 and E2).

Gross lesions related to allyl acetate treatment were observed in the liver, forestomach, and thorax/abdomen of male and female rats in the 100 mg/kg groups. The liver was observed to be pale, granular, and friable at necropsy. The forestomach had a red discoloration, and the thorax/abdomen contained a red fluid.

Microscopically, males administered 12 mg/kg or greater and females in the 25 and 50 mg/kg groups had significantly increased incidences of squamous epithelial hyperplasia in the forestomach compared to those of the vehicle controls (Tables 8, A1 and A2). The incidences of epithelial necrosis, hemorrhage, and inflammation of the forestomach in 100 mg/kg males and females were significantly greater than those in the vehicle controls. Incidences of hemorrhage, inflammation, and epithelial necrosis were also increased in the large and small intestines of male rats in the 100 mg/kg group.

The incidences of periportal hepatocyte hypertrophy in the liver in males and females in the 25, 50, and 100 mg/kg groups were significantly greater than those in the vehicle controls. Males and females in the 50 and 100 mg/kg groups generally had increased incidences of bile duct hyperplasia, hemorrhage, hepatocyte necrosis, periportal hepatocyte hydropic degeneration and mitotic alteration, mineralization, mitotic alteration, hemosiderin pigmentation, and portal fibrosis and granulomatous inflammation compared to those in the vehicle controls. Females in the 25 mg/kg group also had a significantly increased incidence of hemosiderin pigmentation.

Incidences of hyperplasia in bone marrow, hemorrhage in the mediastinal lymph node, lymphoid depletion in the mandibular lymph node, hemorrhage and lymphoid depletion in the mesenteric lymph node, lymphoid follicular cell depletion and hematopoietic cell proliferation of the red pulp in the spleen, and hemorrhage and thymocyte necrosis in the thymus in 100 mg/kg males were significantly increased relative to those in the vehicle controls (Table A1). Incidences of hyperplasia in bone marrow, lymphoid depletion in the mandibular lymph node, hemorrhage in the mesenteric lymph node, lymphoid follicular cell depletion and hematopoietic cell proliferation of the red pulp in the spleen, and hemorrhage and thymocyte necrosis in the thymus in 100 mg/kg females were significantly increased relative to those in the vehicle controls (Table A2).

TABLE 8
Incidence of Selected Nonneoplastic Lesions in Rats in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	6 mg/kg	12 mk/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male						
Forestomach[a]	10	10	10	10	10	10
Epithelium Hyperplasia, Squamous[b]	0	2 (1.0)[c]	6** (1.3)	5* (1.8)	10** (2.0)	4* (1.8)
Epithelium, Necrosis	0	0	0	0	0	9** (3.8)
Hemorrhage	0	0	0	0	0	9** (2.7)
Inflamation, Chronic Active	0	0	0	0	0	9** (2.3)
Liver	10	10	10	10	10	10
Bile Duct, Hyperplasia	0	0	0	1 (1.0)	9** (1.8)	10** (1.8)
Hemorrhage	0	0	0	0	1 (1.0)	9** (2.8)
Hepatocyte, Periportal, Degeneration, Hydropic	0	0	0	0	2 (1.5)	9** (1.7)
Hepatocyte, Periportal, Hypertrophy	0	0	0	5* (1.0)	8** (1.1)	9** (2.6)
Hepatocyte, Necrosis	0	0	0	0	4* (1.5)	10** (3.7)
Hepatocyte, Periportal, Mitotic Alteration	0	0	0	1 (1.0)	3 (1.0)	4* (1.8)
Mineralization	0	0	0	1 (1.0)	0	10** (2.6)
Pigmentation, Hemosiderin	0	0	0	1 (1.0)	6** (1.3)	0
Portal, Fibrosis	0	0	0	0	4* (1.8)	6** (2.3)
Portal, Inflammation Granulomatous	0	0	0	1 (1.0)	4* (1.5)	7** (1.9)
Female						
Forestomach	10	10	10	10	10	9
Epithelium Hyperplasia, Squamous	0	1 (1.0)	3 (1.3)	9** (1.2)	7** (1.3)	1 (2.0)
Epithelium, Necrosis	0	0	0	0	0	9** (3.9)
Hemorrhage	0	0	0	0	0	9** (2.7)
Inflammation, Chronic Active	0	0	0	0	0	5* (2.2)
Liver	10	10	10	10	10	10
Bile Duct, Hyperplasia	0	0	0	1 (1.0)	10** (2.9)	4* (1.8)
Hemorrhage	0	0	0	0	0	10** (3.2)
Hepatocyte, Necrosis	0	0	0	0	2 (1.5)	10** (3.5)
Hepatocyte, Periportal Degeneration, Hydropic	0	0	0	0	7** (1.3)	3 (2.3)
Hepatocyte, Periportal, Hypertrophy	0	0	0	7** (1.1)	10** (2.9)	6** (3.0)
Hepatocyte, Periportal, Mitotic Alteration	0	0	0	2 (1.0)	9** (1.9)	1 (1.0)
Mineralization	0	0	0	0	0	7** (2.3)
Pigmentation, Hemosiderin	0	0	0	6** (1.0)	9** (1.9)	0
Portal, Fibrosis	0	0	0	0	10** (2.4)	2 (2.0)
Portal Inflammation, Granulomatous	0	0	0	3 (1.0)	10** (1.7)	3 (1.0)

* 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 organ 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

Allyl alcohol: Hematology and clinical chemistry data are presented in Table C2. Similar to the allyl acetate study, there were minimal decreases in mean cell volume and increases in platelet counts in 25 mg/kg males. There were no other changes in the hematology data that indicate a treatment-related effect. At week 14, alkaline phosphatase activities were decreased and bile acid concentrations were increased in 12 and 25 mg/kg males and females; increased bile acid concentrations also occurred in the allyl acetate study. In general, increases in alkaline phosphatase activity and bile acid concentration are used as markers of cholestasis. Thus, the increased bile acid concentrations and decreased alkaline phosphatase activities would appear to be incongruous. It has been suggested that decreased alkaline phosphatase activity may be related to altered feed intake (Travlos *et al.*, 1996). In this study, however, there were no changes in mean body weights or body weight gains that would suggest an altered nutritional state. Additionally, serum bile acid concentration can be affected by mechanisms other than cholestasis (e.g., altered enterohepatic circulation); impaired liver function and noncholestatic liver injury can result in increased circulating bile acid concentrations (Hofmann, 1988). Bile duct hyperplasia and periportal hepatocyte hypertrophy were observed microscopically in the livers of 25 mg/kg females and are consistent with increased bile acid concentrations but not the decreased alkaline phosphatase activities. Differences in tissue distribution, changes in cell membrane integrity, and altered enzyme synthesis, release, catabolism, and inhibition have been implicated as effectors of serum enzyme activity (Boyd, 1983; Schmidt and Schmidt, 1987, 1989; Pappas, 1989). Thus, regardless of the morphological liver changes and increased bile acid concentrations, altered enzyme metabolism may, in part, explain the decrease in alkaline phosphatase activities. There were sporadic increases and decreases in various parameters at various time points that, in general, did not demonstrate a treatment relationship and/or were inconsistent between sexes; they were not considered toxicologically relevant.

The absolute liver weights in 25 mg/kg males were significantly greater than those of the vehicle controls (Table D2). The relative liver weights in 6, 12, and 25 mg/kg males were significantly greater than those of the vehicle controls.

No differences were found in sperm motility parameters between dosed and vehicle control males (Table E3). Female rats in the 25 mg/kg group spent more time in diestrus and less time in metestrus than vehicle control females (Table E4).

No treatment-related gross lesions were observed in male or female rats administered allyl alcohol. Microscopically, the incidences of squamous epithelial hyperplasia in the forestomach of males and females in the 6, 12, and 25 mg/kg groups were significantly greater than those in the vehicle controls (Tables 9, A3, and A4). The incidences of bile duct hyperplasia and periportal hepatocyte hypertrophy in the liver in 25 mg/kg females were significantly greater than those in the vehicle controls. One male in the 25 mg/kg group also had both bile duct hyperplasia and periportal hepatocyte hypertrophy. One female in the 25 mg/kg group had hepatocyte necrosis (Tables 9, A3, and A4).

TABLE 9
Incidence of Selected Nonneoplastic Lesions in Rats in the 14-Week Gavage Study of Allyl Alcohol

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Male						
Forestomach[a]	10	10	10	10	10	10
Hyperplasia, Squamous[b]	0	0	0	0	0	1 (1.0)
Epithelium Hyperplasia, Squamous	0	0	0	5* (1.0)[c]	7** (1.0)	6** (1.0)
Liver	10	10	10	10	10	10
Bile Duct, Hyperplasia	0	0	0	0	0	1 (1.0)
Hepatocyte, Periportal, Hypertrophy	0	0	0	0	0	1 (1.0)
Female						
Forestomach	10	10	10	9	10	10
Epithelium, Hyperplasia, Squamous	0	0	1 (1.0)	4* (1.0)	9** (1.0)	8** (1.0)
Liver	10	10	10	9	10	10
Bile Duct, Hyperplasia	0	0	0	0	0	8** (1.1)
Hepatocyte, Periportal, Hypertrophy	0	0	0	0	0	8** (1.1)
Hepatocyte, Necrosis	0	0	0	0	0	1 (2.0)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with organ 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

Acrolein: Hematology and clinical chemistry data are presented in Table C3, and selected data are presented in Table 10. Numerous changes in the hematology and clinical chemistry variables occurred in response to acrolein administration. Similar to the allyl acetate and allyl alcohol studies, platelet counts were significantly increased in 5 and 10 mg/kg rats on day 23 and at the end of the study.

On day 4, the hematocrit values, hemoglobin concentrations, and erythrocyte counts were generally significantly increased in 10 mg/kg males and females; these increases are consistent with an erythrocytosis. Because there was a marked decrease in body weights in 10 mg/kg animals compared to the vehicle controls after 2 weeks of acrolein administration, and because it is generally considered that rats that do not eat also do not drink, the increased red cell mass would be consistent with an altered hydration status. Albumin concentrations are also affected by hydration status and, in this study, would have been expected to increase on day 4. That did not happen and, in fact, albumin concentrations were generally decreased in a dose-related fashion in the 2.5 mg/kg or greater groups. Because liver function and nutritional status influence albumin, the decreases in albumin concentrations suggest an altered nutritional status and/or hepatotoxicity. On day 4, total protein concentrations were also decreased in 10 mg/kg rats; these decreases would be consistent with the decreased albumin concentrations. Decreased albumin and total protein concentrations also occurred on day 4 in the allyl acetate study.

On day 23, the increased erythron occurred in the 5 and 10 mg/kg groups. By week 14, however, the erythrocytosis was only evident in the surviving 10 mg/kg females and was replaced by a decreased red cell mass in the surviving 10 mg/kg male. On day 23 and at week 14, the erythron changes were accompanied by increased reticulocyte counts in the 5 and 10 mg/kg males and females, suggesting an increase in erythropoiesis in these groups. Increased reticulocyte counts in the event of a normal or increased red cell mass would appear to be inappropriate; however, the increased reticulocyte count in the surviving 10 mg/kg male at week 14 was consistent with a regenerative response to the anemia. Additionally, microscopic evidence of gastric hemorrhage in 5 mg/kg males and 10 mg/kg males and females suggests that there was an underlying blood loss resulting in an increase in red cell production.

On days 4 and 23, the increased erythron was accompanied by slight decreases in mean cell volumes and mean cell hemoglobin values in 10 mg/kg rats suggesting that the circulating erythrocytes were smaller than expected. The microcytosis could suggest an ineffective erythropoiesis due to change in iron metabolism and a subsequent alteration in heme production (Jain, 1986). Conditions such as acute phase reactions and anemias of chronic disorders have been shown to alter iron availability for erythropoiesis (Smith, 1989). In these studies, there was evidence of a gastric inflammatory process, and this may have contributed to an ineffective erythropoiesis by altering the availability of iron for red cell production. At week 14, the mean cell volume and mean cell hemoglobin value were increased in the 5 and 10 mg/kg males, consistent with the increased numbers of larger reticulocytes.

TABLE 10
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Acrolein[a]

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	9	10	9
Day 23	10	10	10	9	10	7
Week 14	10	10	9	7	8	1[b]
Hematocrit (%)						
Day 4	43.9 ± 0.5	43.8 ± 0.5	43.1 ± 0.8	43.7 ± 0.6	44.9 ± 0.9	48.3 ± 1.2**
Day 23	44.7 ± 0.3	44.8 ± 0.4	45.2 ± 0.5	45.6 ± 0.5	47.8 ± 1.2**	46.1 ± 1.9
Week 14	46.5 ± 0.4	47.5 ± 0.3	46.6 ± 0.5	47.1 ± 0.3	48.4 ± 0.5*	33.0
Hemoglobin (g/dL)						
Day 4	13.8 ± 0.1	13.9 ± 0.2	13.8 ± 0.2	13.9 ± 0.2	14.3 ± 0.3	15.7 ± 0.3**
Day 23	14.7 ± 0.1	14.7 ± 0.1	14.8 ± 0.1	14.8 ± 0.2	15.5 ± 0.3	14.7 ± 0.6
Week 14	15.1 ± 0.2	15.2 ± 0.1	14.9 ± 0.1	15.2 ± 0.1	15.6 ± 0.3	10.5
Erythrocytes (10^6/µL)						
Day 4	7.18 ± 0.09	7.19 ± 0.09	7.07 ± 0.13	7.22 ± 0.11	7.45 ± 0.13	8.24 ± 0.18**
Day 23	7.31 ± 0.05	7.35 ± 0.07	7.34 ± 0.10	7.43 ± 0.13	7.82 ± 0.18*	8.09 ± 0.30*
Week 14	8.61 ± 0.07	8.71 ± 0.08	8.57 ± 0.12	8.64 ± 0.09	8.69 ± 0.10	5.44
Reticulocytes (10^6/µL)						
Day 4	0.31 ± 0.02	0.34 ± 0.02	0.32 ± 0.02	0.30 ± 0.03	0.33 ± 0.03	0.30 ± 0.02
Day 23	0.16 ± 0.01	0.17 ± 0.02	0.14 ± 0.01	0.18 ± 0.02	0.20 ± 0.02*	0.38 ± 0.04**
Week 14	0.16 ± 0.01	0.15 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.20 ± 0.02	0.85
Mean cell volume (fL)						
Day 4	61.2 ± 0.2	61.1 ± 0.2	61.2 ± 0.4	60.7 ± 0.4	60.4 ± 0.3	58.4 ± 0.4**
Day 23	61.3 ± 0.2	61.0 ± 0.3	61.6 ± 0.3	61.3 ± 0.6	61.2 ± 0.4	56.9 ± 0.8**
Week 14	53.8 ± 0.2	54.5 ± 0.2*	54.3 ± 0.4	54.6 ± 0.3	55.8 ± 0.2**	61.0
Mean cell hemoglobin (pg)						
Day 4	19.3 ± 0.1	19.4 ± 0.1	19.5 ± 0.1	19.3 ± 0.1	19.2 ± 0.1	19.1 ± 0.1
Day 23	20.1 ± 0.1	19.9 ± 0.1	20.2 ± 0.1	19.9 ± 0.2	19.9 ± 0.2	18.1 ± 0.3**
Week 14	17.5 ± 0.1	17.5 ± 0.1	17.4 ± 0.2	17.6 ± 0.1	18.0 ± 0.1*	19.3
Platelets (10^3/µL)						
Day 4	918.4 ± 17.8	906.1 ± 23.0	914.4 ± 32.9	980.0 ± 24.5*	1,043.1 ± 33.3**	1,172.4 ± 38.4**
Day 23	743.4 ± 7.1	747.3 ± 16.3	737.4 ± 9.2	729.7 ± 21.3	832.1 ± 14.0**	980.7 ± 59.8**
Week 14	626.4 ± 11.6	648.4 ± 11.2	645.8 ± 10.9	651.4 ± 14.6	754.6 ± 14.3**	1,186.0
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	9
Day 23	10	10	10	9	10	7
Week 14	10	10	10	8	8	1
Urea nitrogen (mg/dL)						
Day 4	9.8 ± 0.4	10.2 ± 0.3	10.6 ± 0.5	10.2 ± 0.4	11.7 ± 0.7*	12.9 ± 1.2**
Day 23	11.8 ± 0.4	12.4 ± 0.4	11.5 ± 0.5	12.6 ± 0.4	15.5 ± 1.1**	15.6 ± 1.6**
Week 14	15.4 ± 0.5	15.4 ± 0.3	15.2 ± 0.4	18.0 ± 0.4**	18.1 ± 0.7**	16.0
Total Protein (g/dL)						
Day 4	5.8 ± 0.0	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.5 ± 0.1	4.6 ± 0.1**
Day 23	6.0 ± 0.0	6.0 ± 0.0	6.1 ± 0.0	5.8 ± 0.0**	5.4 ± 0.2**	5.0 ± 0.3**
Week 14	6.8 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.5 ± 0.1**	5.9 ± 0.2**	5.1

TABLE 10
Hematology and Clinical Chemistry Data for Rats on the 14-Week Gavage Study of Acrolein

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	9
Day 23	10	10	10	9	10	7
Week 14	10	10	10	8	8	1
Albumin (g/dL)						
Day 4	4.3 ± 0.0	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1*	3.9 ± 0.1**	3.2 ± 0.1**
Day 23	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.2 ± 0.1**	3.9 ± 0.2**	3.6 ± 0.2**
Week 14	4.8 ± 0.1	4.8 ± 0.0	4.8 ± 0.0	4.7 ± 0.1	4.2 ± 0.1**	3.6
Alkaline phosphatase (IU/L)						
Day 4	1,628 ± 27	1,667 ± 47	1,648 ± 33	1,476 ± 31*	1,187 ± 73**	750 ± 71**
Day 23	1,189 ± 25	1,212 ± 23	1,214 ± 28	1,093 ± 22*	923 ± 32**	760 ± 59**
Week 14	628 ± 17	663 ± 13	593 ± 15	620 ± 14	479 ± 19**	440
Female						
Hematology						
n						
Day 4	10	9	9	10	10	10
Day 23	10	10	10	10	10	6
Week 14	9	10	7	7	9	2
Hematocrit (%)						
Day 4	47.2 ± 1.0	46.0 ± 1.3	45.3 ± 0.9	45.7 ± 0.9	46.9 ± 0.7	52.2 ± 1.4
Day 23	46.5 ± 0.5	47.3 ± 0.6	46.7 ± 0.4	47.8 ± 0.5	50.1 ± 0.7**	49.1 ± 1.1*
Week 14	46.8 ± 0.3	46.1 ± 0.5	47.8 ± 0.3	47.2 ± 0.4	48.4 ± 0.8	49.6 ± 7.8
Hemoglobin (g/dL)						
Day 4	14.8 ± 0.3	14.7 ± 0.4	14.2 ± 0.2	14.6 ± 0.2	14.9 ± 0.3	16.7 ± 0.5**
Day 23	14.8 ± 0.1	15.1 ± 0.2	14.9 ± 0.1	15.2 ± 0.2	15.9 ± 0.2**	15.3 ± 0.4*
Week 14	15.0 ± 0.1	14.9 ± 0.1	15.2 ± 0.1	15.0 ± 0.1	15.6 ± 0.3	15.9 ± 2.6
Erythrocytes (10^6/μL)						
Day 4	7.55 ± 0.17	7.45 ± 0.21	7.35 ± 0.14	7.39 ± 0.12	7.65 ± 0.14	8.68 ± 0.23**
Day 23	7.43 ± 0.09	7.63 ± 0.10	7.53 ± 0.06	7.68 ± 0.09	8.09 ± 0.12**	8.12 ± 0.25**
Week 14	8.04 ± 0.06	7.96 ± 0.07	8.23 ± 0.04	8.07 ± 0.05	8.31 ± 0.11	8.19 ± 1.52
Reticulocytes (10^6/μL)						
Day 4	0.24 ± 0.02	0.24 ± 0.02	0.26 ± 0.01	0.26 ± 0.02	0.24 ± 0.02	0.29 ± 0.03
Day 23	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.32 ± 0.04**
Week 14	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.16 ± 0.02**	0.38 ± 0.07**
Mean cell volume (fL)						
Day 4	62.4 ± 0.3	61.9 ± 0.3	61.6 ± 0.3*	61.8 ± 0.5	61.5 ± 0.3	60.1 ± 0.4**
Day 23	62.5 ± 0.3	61.9 ± 0.2	62.2 ± 0.3	62.3 ± 0.3	61.8 ± 0.2	60.7 ± 0.8*
Week 14	58.3 ± 0.2	57.9 ± 0.2	58.3 ± 0.2	58.6 ± 0.3	58.3 ± 0.2	61.0 ± 2.0
Mean cell hemoglobin (pg)						
Day 4	19.6 ± 0.1	19.8 ± 0.1	19.4 ± 0.1	19.8 ± 0.1	19.5 ± 0.1	19.3 ± 0.1
Day 23	20.0 ± 0.2	19.9 ± 0.1	19.9 ± 0.2	19.9 ± 0.1	19.6 ± 0.1	18.9 ± 0.2**
Week 14	18.7 ± 0.1	18.7 ± 0.1	18.5 ± 0.1	18.6 ± 0.1	18.8 ± 0.1	19.4 ± 0.5
Platelets (10^3/μL)						
Day 4	942.9 ± 18.8	940.4 ± 18.5	987.7 ± 32.1	989.5 ± 32.0*	1,083.8 ± 35.5**	1,113.4 ± 32.5**
Day 23	760.8 ± 14.9	767.1 ± 17.7	750.5 ± 9.0	795.1 ± 9.0*	890.7 ± 20.9**	1,084.2 ± 45.2**
Week 14	652.4 ± 9.8	645.1 ± 14.5	646.4 ± 16.7	675.3 ± 20.1	770.8 ± 9.5**	1,098.5 ± 117.5*

TABLE 10
Hematology and Clinical Chemistry Data for Rats on the 14-Week Gavage Study of Acrolein

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Female (continued)						
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	10	9	10	10	10	6
Week 14	10	10	9	8	9	2
Urea nitrogen (mg/dL)						
Day 4	12.8 ± 0.7	13.1 ± 0.4	11.8 ± 0.6	12.9 ± 0.5	14.3 ± 0.5	14.2 ± 1.7
Day 23	14.1 ± 0.3	14.3 ± 0.4	14.4 ± 0.6	14.0 ± 0.4	15.6 ± 1.0	15.3 ± 1.2
Week 14	14.8 ± 0.5	16.6 ± 0.3**	16.4 ± 0.6*	18.6 ± 0.6**	19.9 ± 1.0**	22.5 ± 2.5**
Total protein (g/dL)						
Day 4	6.0 ± 0.1	5.9 ± 0.1	5.9 ± 0.0	5.8 ± 0.1	6.1 ± 0.1	4.5 ± 0.2**
Day 23	6.1 ± 0.0	6.3 ± 0.2	6.1 ± 0.0	5.9 ± 0.1	5.7 ± 0.1**	5.5 ± 0.2**
Week 14	6.7 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.4 ± 0.2*	6.1 ± 0.1**	5.0 ± 0.4**
Albumin (g/dL)						
Day 4	4.6 ± 0.1	4.6 ± 0.1	4.5 ± 0.0	4.4 ± 0.0*	4.5 ± 0.1	3.1 ± 0.1**
Day 23	4.5 ± 0.0	4.7 ± 0.1	4.6 ± 0.0	4.4 ± 0.1	4.1 ± 0.1**	3.9 ± 0.1**
Week 14	5.0 ± 0.1[c]	4.8 ± 0.0	5.0 ± 0.1	4.8 ± 0.1	4.6 ± 0.0**	3.6 ± 0.3**
Alkaline phosphatase (IU/L)						
Day 4	1,280 ± 37	1,202 ± 23	1,225 ± 22	1,145 ± 27**	1,005 ± 41**	559 ± 61**
Day 23	880 ± 14	937 ± 66	832 ± 13	804 ± 20**	663 ± 39**	656 ± 18**
Week 14	470 ± 14	495 ± 19	470 ± 18	510 ± 24	373 ± 19**	318 ± 51**

* 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] No standard error was calculated because less than two measurements were available.
[c] n=9

A neutrophilia, evidenced by increased segmented neutrophil counts, occurred in 5 and 10 mg/kg males and females. The neutrophilia was most pronounced on day 4 and ameliorated with time. Though no lesions were observed in the 5 mg/kg rats, the neutrophilia would be consistent with an acute inflammatory process that resolved with time and was supported by the necrosis and inflammation observed microscopically in the stomach of the 10 mg/kg rats. Increased leukocyte counts also occurred in 5 and 10 mg/kg males and females at the same time points and would be consistent with the increased neutrophil counts.

On day 23 and at week 14, the albumin and total protein concentrations remained decreased in 5 and 10 mg/kg males and females, consistent with altered nutrition and/or liver function. At week 14, urea nitrogen concentrations were increased in 2.5 mg/kg or greater males and all dosed groups of females, suggesting a possible decrease in glomerular filtration. It is known, however, that urea nitrogen concentration can be influenced by many extrarenal factors (e.g., high protein diets, dehydration, liver function, animal health, and nutritional status) (Finco, 1989). Because creatinine concentration, another marker of kidney function, was unchanged in this study, the urea nitrogen concentration increases were probably related to a nonrenal effect.

Decreases in alkaline phosphatase activities generally occurred at all time points in 2.5 mg/kg or greater males and females. The decreases were most pronounced on day 4 and ameliorated with time, involving only the 5 and 10 mg/kg groups at week 14. Decreased alkaline phosphatase activity also occurred in the allyl alcohol study. It has been suggested that decreased alkaline phosphatase activity may be related to altered feed intake (Travlos *et al.*, 1996). In this study, there were marked differences in body weight gains of 10 mg/kg rats compared to those of the vehicle controls. Altered nutrition does not, however, explain the decreases in the 2.5 and 5 mg/kg groups; thus, altered enzyme metabolism may, in part, explain the decreases in alkaline phosphatase activities in this study.

At week 14, the surviving 10 mg/kg male had an alanine aminotransferase activity that was approximately twofold greater than that of the vehicle control group, suggesting a heptocellular effect. However, sorbitol dehydrogenase activity, another marker of hepatocellular leakage, was decreased in this male. It has been demonstrated that glucocorticoids can increase liver alanine aminotransferase in a dose-related manner by as much as 14-fold (Rosen *et al.*, 1959a,b). Thus, increases in serum alanine aminotransferase activity but not sorbitol dehydrogenase activity are consistent with a compound-induced or treatment-related, stress-induced increase in liver alanine aminotransferase. Because this animal was the only survivor in the 10 mg/kg male group, weighed significantly less than the vehicle control males, and had an anemia, treatment-related stress could be postulated as the cause of the increased alanine aminotransferase activity. This animal also had a markedly decreased lymphocyte count consistent with a stress-related response. There were sporadic increases and decreases in various parameters at various time points that, in general, did not demonstrate a treatment relationship and/or were inconsistent between males and females; they were not considered toxicologically relevant.

The absolute and relative liver weights of 5 and 10 mg/kg females were significantly greater than those of the vehicle controls (Table D3). The absolute and relative thymus weights of 10 mg/kg females and the absolute heart weights of 5 and 10 mg/kg females were significantly less than those of the vehicle controls.

Gross lesions related to acrolein treatment were observed in the forestomach and glandular stomach of male and female rats, primarily in the 10 mg/kg groups, and consisted of red or white discoloration. Microscopically, males in the 5 and 10 mg/kg groups and females in the 2.5, 5, and 10 mg/kg groups had increased incidences of squamous epithelial hyperplasia in the forestomach relative to those of the vehicle controls (Tables 11, A5, and A6). Incidences of hemorrhage in the glandular stomach of males and females in the 10 mg/kg groups were significantly greater than those in the vehicle controls.

Incidences of hyperplasia in bone marrow, lymphoid follicular cell depletion in the spleen, and inflammation in the nose of 10 mg/kg males and females and thymacyte necrosis in 10 mg/kg males were significantly greater than those in the vehicle controls (Tables A5 and A6).

TABLE 11
Incidence of Selected Nonneoplastic Lesions in Rats in the 14-Week Gavage Study of Acrolein

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Male						
Forestomach[a]	10	10	10	10	10	10
Epithelium, Hyperplasia, Squamous[b]	0	0	0	3 (1.0)[c]	8** (1.0)	9** (1.6)
Glandular Stomach	10	10	10	10	10	10
Hemorrhage	0	0	0	0	3 (1.0)	5* (1.8)
Epithelium, Necrosis	0	0	0	0	0	1 (2.0)
Female						
Forestomach	10	10	10	10	10	10
Epithelium, Hyperplasia, Squamous	0	0	3 (1.0)	5* (1.0)	8** (1.0)	10** (2.7)
Glandular Stomach	10	10	10	10	10	10
Hemorrhage	0	0	0	0	0	4* (1.8)
Inflammation, Chronic Active	0	0	0	0	0	1 (1.0)
Epithelium, Necrosis	0	0	0	0	0	1 (2.0)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with organ 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

MICE

Allyl acetate: All males and females in the 125 mg/kg group died during the first week of the study. All other early deaths, except five in the 62.5 mg/kg male group and one in the 32 mg/kg female group, were gavage accidents (Table 12). Final mean body weights and mean body weight gains of males and females were similar to those of the vehicle controls (Table 12 and Figure 5). Clinical findings included lethargy, abnormal breathing, thinness, and ruffled fur among mice that died early.

Allyl alcohol: One 50 mg/kg female died due to gavage accident; all other animals survived to the end of the study (Table 13). Final mean body weights of dosed male and female mice were similar to those of the vehicle controls. The mean body weight gain of males administered 50 mg/kg was significantly less than that of the vehicle controls; the mean body weight gains in all other male and female dosed groups were similar to those of the vehicle control groups (Table 13 and Figure 6). No clinical findings were evident in dosed mice.

Acrolein: All males and females administered 20 mg/kg died during the first week of the study (Table 14). All other early deaths, except one male and one female administered 10 mg/kg, were unrelated to chemical administration. Final mean body weights and mean body weight gains of all dosed groups were generally similar to those of the vehicle control groups (Table 14 and Figure 7). No clinical findings were evident in dosed mice.

The concentrations of 3-hydroxy mercapturic acid in the urine of mice after one or 45 doses of allyl acetate or allyl alcohol increased linearly with dose (Tables F4 and F5). In mice dosed with acrolein, the concentrations increased nonlinearly with dose at the first time point and linearly with dose at the second time point (except at 10 mg/kg) (Table F6).

TABLE 12
Survival and Body Weight of Mice in the 14-Week Gavage Study of Allyl Acetate

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	9/10[c]	26.5 ± 0.4	36.3 ± 1.1	9.8 ± 0.8	
8	7/10[d]	26.7 ± 0.6	38.1 ± 0.8	11.3 ± 0.6	105
16	10/10	26.2 ± 0.4	36.6 ± 0.8	10.4 ± 0.6	101
32	9/10[e]	26.0 ± 0.3	36.8 ± 0.8	10.8 ± 0.7	101
62.5	2/10[f]	26.1 ± 0.2	36.2 ± 0.9	10.9 ± 1.0	100
125	0/10[c]	25.9 ± 0.4	—	—	—
Female					
0	9/10[e]	20.5 ± 0.4	30.7 ± 0.6	10.1 ± 0.5	
8	8/10[c]	20.6 ± 0.2	31.9 ± 0.4	11.1 ± 0.5	104
16	6/10[g]	20.8 ± 0.3	32.7 ± 1.7	11.7 ± 1.4	107
32	7/10[h]	20.5 ± 0.3	31.3 ± 1.1	10.9 ± 1.1	102
62.5	6/10[g]	20.8 ± 0.2	29.7 ± 0.9	9.1 ± 0.8	97
125	0/10[c]	20.3 ± 0.2	—	—	—

[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] Week of death: 1
[d] Week of death: 2, 3, 3
[e] Week of death: 2
[f] Week of death: 1, 1, 2, 11, 11, 11, 11, 12
[g] Week of death: 1, 1, 1, 2
[h] Week of death: 1, 1, 2

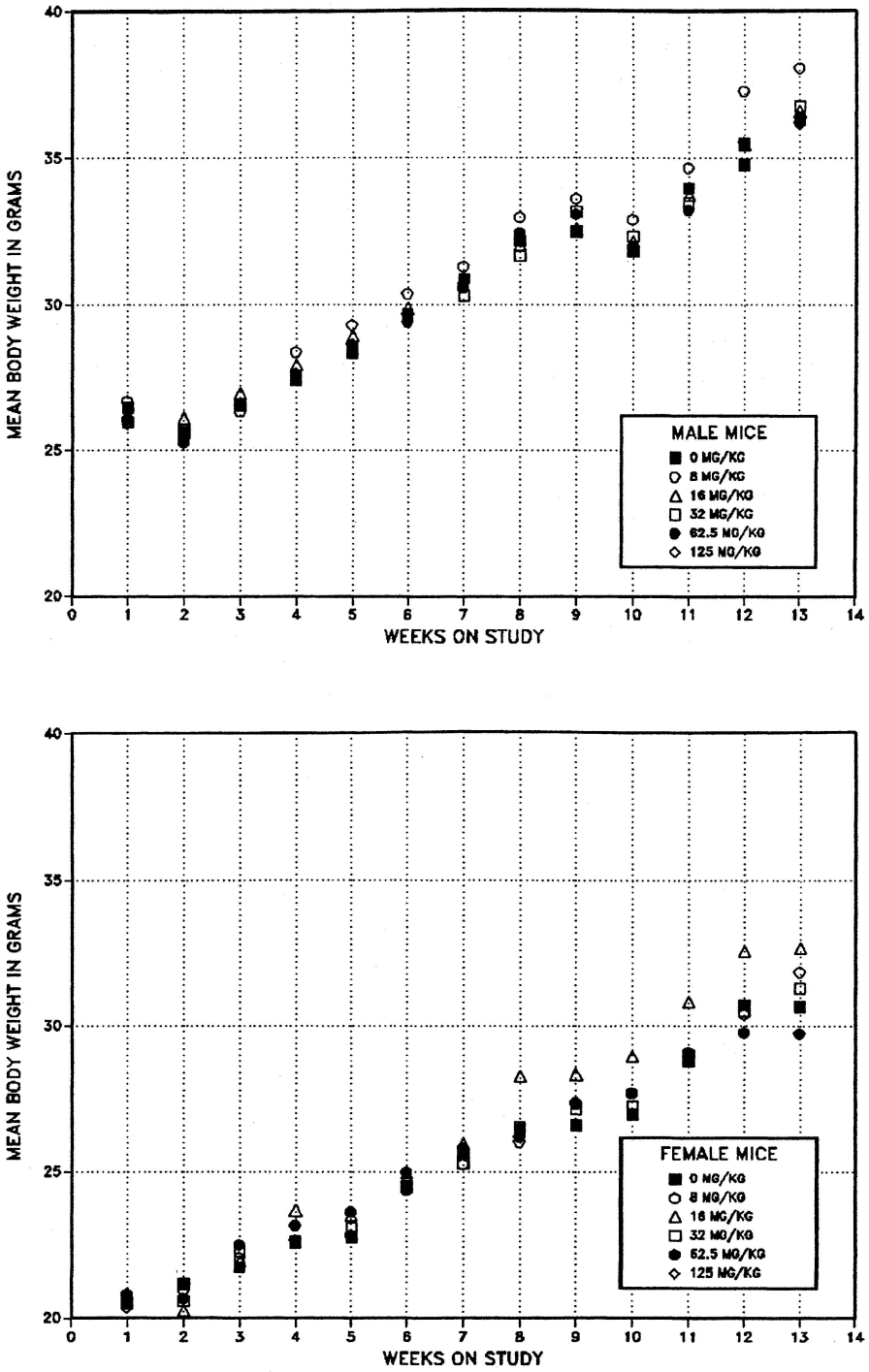


FIGURE 5
Body Weights of Mice Administered Allyl Acetate by Gavage for 14 Weeks

TABLE 13
Survival and Body Weights of Mice in the 14-Week Gavage Study of Allyl Alcohol

Dose (mg/kg)	Survival[a]	Mean Body Weight[b]			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	25.8 ± 0.4	40.9 ± 1.1	15.1 ± 0.7	
3	10/10	25.4 ± 0.5	39.5 ± 1.2	14.1 ± 1.0	97
6	10/10	25.6 ± 0.5	40.3 ± 0.9	14.8 ± 0.8	99
12	10/10	25.7 ± 0.4	39.4 ± 1.0	13.7 ± 0.8	96
25	10/10	25.4 ± 0.4	39.1 ± 1.0	13.7 ± 0.8	96
50	10/10	25.3 ± 0.4	37.9 ± 0.5	12.6 ± 0.5*	93
Female					
0	10/10	19.3 ± 0.2	31.7 ± 1.2	12.4 ± 1.0	
3	10/10	18.9 ± 0.3	33.0 ± 1.3	14.2 ± 1.3	104
6	10/10	20.3 ± 0.4	34.8 ± 1.5	14.5 ± 1.3	110
12	10/10	20.4 ± 0.2*	33.9 ± 1.0	13.5 ± 1.0	107
25	10/10	19.5 ± 0.3	32.5 ± 1.3	13.0 ± 1.1	103
50	9/10[c]	19.8 ± 0.4	31.4 ± 1.1	11.7 ± 0.9	99

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 14 weeks/number initially in group. Subsequent calculations are based on animals surviving to the end of the study.
[b] Weights and weight changes are given as mean ± standard error.
[c] Week of death: 1

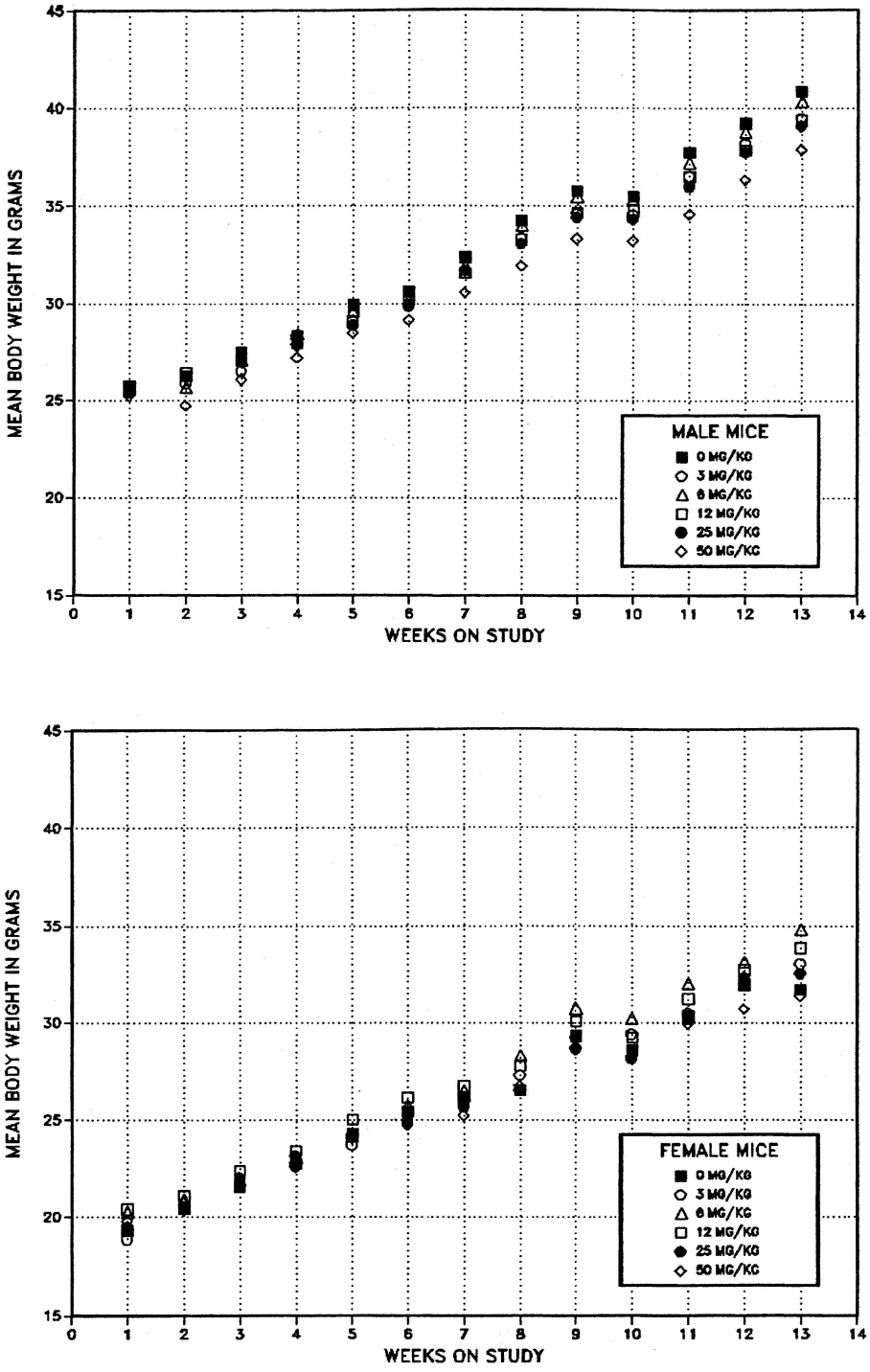


FIGURE 6
Body Weights of Mice Administered Allyl Alcohol by Gavage for 14 Weeks

TABLE 14
Survival and Body Weights of Mice in the 14-Week Gavage Study of Acrolein

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	25.8 ± 0.3	35.6 ± 1.2	9.8 ± 0.9	
1.25	9/10[c]	25.5 ± 0.3	36.1 ± 1.0	10.6 ± 0.7	102
2.5	10/10	25.7 ± 0.3	37.7 ± 1.0	12.0 ± 0.8	106
5	9/10[c]	25.7 ± 0.3	36.5 ± 0.5	10.6 ± 0.3	102
10	9/10[d]	26.0 ± 0.3	34.3 ± 1.0	8.2 ± 0.9	96
20	0/10[e]	25.6 ± 0.3	—	—	—
Female					
0	9/10[f]	21.2 ± 0.6	29.8 ± 0.8	8.6 ± 0.7	
1.25	10/10	21.0 ± 0.5	33.6 ± 1.6	12.6 ± 1.3*	113
2.5	10/10	20.8 ± 0.3	31.5 ± 1.3	10.7 ± 1.2	106
5	9/10[g]	20.6 ± 0.3	27.1 ± 0.6	6.5 ± 0.6	91
10	8/10[h]	20.5 ± 0.4	28.5 ± 0.6	8.0 ± 0.5	96
20	0/10[e]	20.5 ± 0.2	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by 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] Week of death: 8
[d] Week of death: 2
[e] Week of death: 1
[f] Week of death: 12
[g] Week of death: 7 (missing)
[h] Week of death: 2, 8

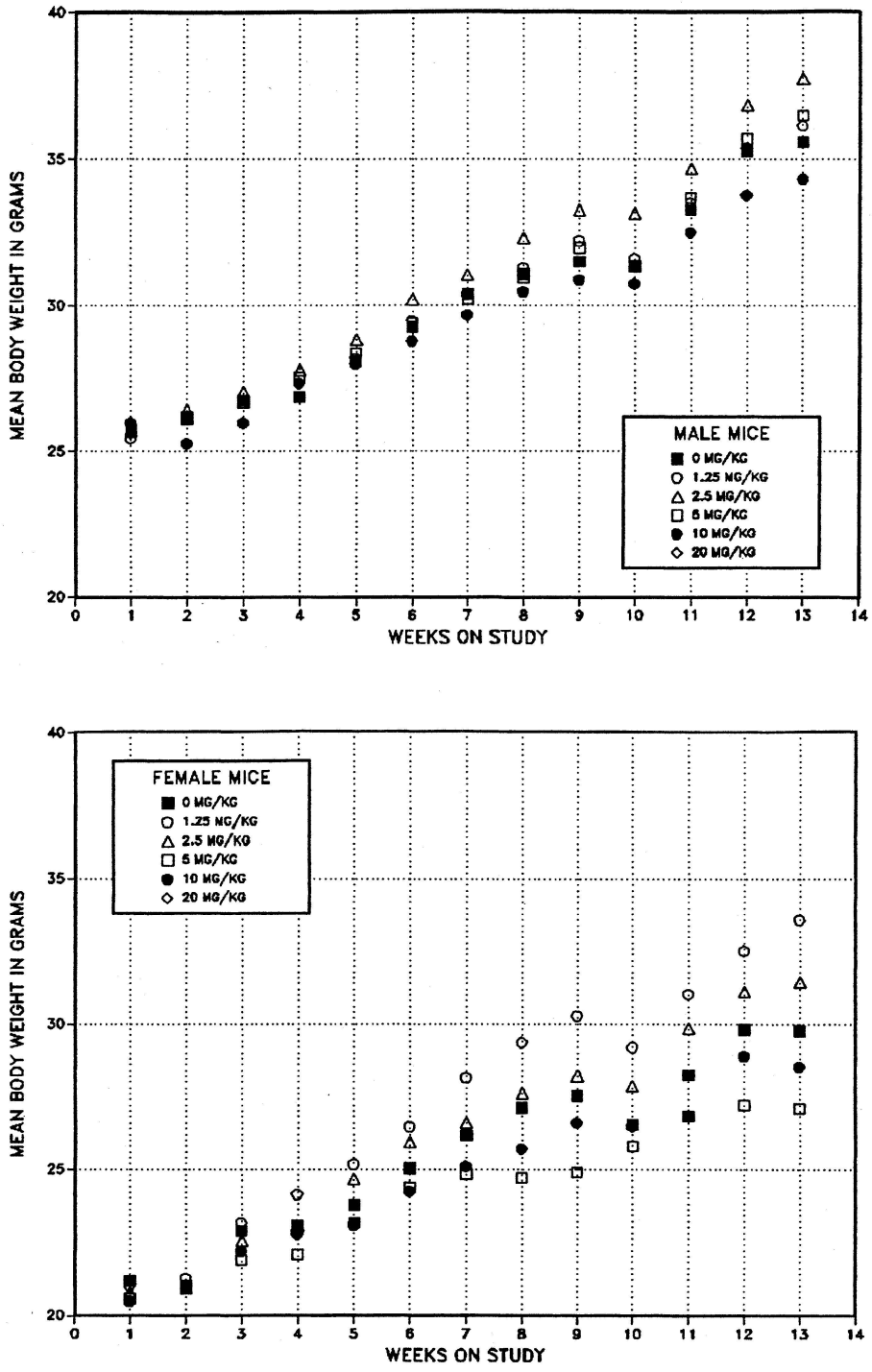


FIGURE 7
Body Weights of Mice Administered Acrolein by Gavage for 14 Weeks

Allyl acetate: There were no biologically significant differences in hematology parameters between dosed and vehicle control mice (Table C4). The absolute and relative organ weights in mice administered allyl acetate were generally similar to those of the vehicle controls (Table D4). No differences were found in sperm motility parameters between dosed and vehicle control mice (Table E5). The estrous cycles of female mice dosed with 16 or 32 mg/kg allyl acetate were significantly longer than that of the vehicle controls (Table E6).

Gross lesions related to allyl acetate treatment included red discoloration in the forestomach and granular, mottled livers in male and female mice and red foci in the glandular stomach of a male mouse. Microscopically, males in the 32 and 62.5 mg/kg groups and females in the 16, 32, and 62.5 mg/kg groups had significantly increased incidences of squamous epithelial hyperplasia in the forestomach compared to those of the vehicle controls (Tables 15, B1, and B2). The incidence of hemorrhage of the forestomach in 125 mg/kg males was significantly greater than that in the vehicle controls. Males in the 62.5 group and males and females in the 125 mg/kg group had significantly increased incidences of hemorrhage in the glandular stomach compared to those in the vehicle controls. In general, males in the 62.5 and 125 mg/kg groups had increased incidences of fibrosis, hepatocyte necrosis, and mineralization in the liver. The incidences of portal cytoplasmic vacuolization of the liver in 62.5 mg/kg males and females were significantly greater than those in the vehicle control groups as was the incidence of hepatocyte necrosis in 125 mg/kg females.

Incidences of necrosis in the mandibular and mesenteric lymph nodes, lymphoid follicular necrosis in the spleen, and necrosis in the thymus in dosed groups were generally increased in 16 mg/kg females and in 62.5 and 125 mg/kg males and females relative to those in the vehicle controls (Tables B1 and B2).

TABLE 15
Incidence of Selected Nonneoplastic Lesions in Mice in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	8 mg/kg	16 mg/kg	32 mg/kg	62.5 mg/kg	125 mg/kg
Male						
Forestomach[a]	10	10	10	10	10	10
Epithelium, Hyperplasia, Squamous[b]	0	0	0	4* (1.5)[c]	10** (2.8)	1 (3.0)
Epithelium, Necrosis	0	0	0	0	0	1 (4.0)
Hemorrhage	0	0	0	0	1 (4.0)	5* (1.4)
Inflammation, Chronic Active	0	0	0	0	1 (2.0)	1 (3.0)
Glandular Stomach	10	10	10	10	10	10
Epithelium, Necrosis	0	0	0	0	0	1 (1.0)
Hemorrhage	0	0	0	0	4* (1.8)	8** (1.5)
Liver	10	10	10	10	10	10
Bile Duct, Hyperplasia	0	0	0	0	2 (2.0)	1 (3.0)
Fibrosis	0	0	0	0	5* (1.6)	1 (2.0)
Hepatocyte, Necrosis	0	0	0	0	5* (2.2)	10** (2.5)
Mineralization	0	0	0	0	5* (1.4)	1 (3.0)
Pigmentation, Hemosiderin	0	0	0	0	3 (1.3)	0
Portal Vacuolization Cytoplasmic	0	0	0	0	6** (1.0)	0
Female						
Forestomach	10	10	10	10	10	10
Epithelium, Hyperplasia, Squamous	0	0	4* (1.5)	5* (1.2)	8** (1.4)	1 (2.0)
Epithelium, Necrosis	0	0	0	0	0	1 (4.0)
Inflammation, Chronic Active	0	0	0	0	0	1 (3.0)
Glandular Stomach	10	10	10	10	10	10
Hemorrhage	0	0	0	0	0	4* (1.0)
Liver	10	10	10	10	10	10
Hepatocyte, Necrosis	0	0	0	0	0	10** (2.1)
Mineralization	0	0	0	0	0	1 (2.0)
Pigmentation, Hemosiderin	0	0	0	0	2 (1.5)	0
Portal, Vacuolization Cytoplasmic	0	0	0	3 (1.0)	4* (1.3)	0

* Significantly different ($P \leq 0.05$) from the vehicle control by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with organ 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

Allyl alcohol: Minimal increases in platelet counts occurred in 50 mg/kg males and in all dosed groups of females; the increases in females were not dose related (Table C5). There were no biologically significant differences in organ weights between dosed and vehicle control mice (Table D5). No differences were found in sperm motility or vaginal cytology parameters between dosed and vehicle control mice (Tables E7 and E8).

No treatment-related gross lesions were observed. Microscopically, males and females in the 12, 25, and 50 mg/kg groups had significantly increased incidences of squamous epithelial hyperplasia in the forestomach compared to those of the vehicle controls (Tables 16, B3, and B4). Incidences of portal cytoplasmic vacuolization in the liver were significantly increased in 50 mg/kg males and females and 25 mg/kg females relative to those of the vehicle controls. One male and one female in the 50 mg/kg groups had hemosiderin pigmentation; one 50 mg/kg female also had granulomatous inflammation and hepatocyte necrosis (Tables B3 and B4).

TABLE 16
Incidence of Selected Nonneoplastic Lesions in Mice in the 14-Week Gavage Study of Allyl Alcohol

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg
Male						
Forestomach[a]	10	10	10	10	10	10
Epithelium Hyperplasia, Squamous[b]	0	1 (1.0)[c]	3 (1.0)	9** (1.1)	10** (2.0)	10** (1.9)
Liver	10	10	10	10	10	10
Portal, Vacuolization Cytoplasmic	0	0	0	0	2 (1.0)	10** (1.0)
Female						
Forestomach	10	10	10	10	10	10
Epithelium, Hyperplasia, Squamous	0	0	0	8** (1.1)	10** (2.0)	9** (2.0)
Liver	10	10	10	10	10	10
Portal, Vacuolization Cytoplasmic	1 (1.0)	1 (1.0)	1 (1.0)	5 (1.0)	8** (1.0)	9** (1.2)

** Significantly different ($P \leq 0.01$) from the vehicle control group by the Fisher exact test
[a] Number of animals with organ 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

Acrolein: At week 14, there were minimal increases in hematocrit values, hemoglobin concentrations, and erythrocyte counts in 10 mg/kg males and in 2.5 mg/kg or greater females (Table C6); in females, the increases were dose related. The mechanism for the increases in the circulating red cell mass is unknown, but an increased erythron did occur in the companion acrolein rat study. Platelet counts were significantly increased in 10 mg/kg males. Platelet count increases also occurred in other companion studies (allyl acetate rat study, allyl alcohol rat and mouse studies, acrolein rat study).

The absolute and relative liver weights of 10 mg/kg males were significantly greater than those of the vehicle controls (Table D6).

Gross lesions related to acrolein treatment included red or white discoloration in the forestomach and glandular stomach of female mice in the 20 mg/kg group. Microscopically, males and females in the 2.5, 5, and 10 mg/kg groups had significantly increased incidences of squamous epithelial hyperplasia in the forestomach compared to those of the vehicle controls (Tables 17, B5, and B6). Incidences of hemorrhage in the glandular stomach in 20 mg/kg males and females significantly exceeded those in the vehicle controls. The incidences of epithelial necrosis and chronic active inflammation in the glandular stomach in 20 mg/kg females were significantly greater than those in the vehicle controls.

Incidences of necrosis in the mandibular and mesenteric lymph nodes, depletion of the lymphoid follicle in the spleen, and necrosis in the thymus in 20 mg/kg males and females were significantly greater than those in the vehicle controls (Tables A5 and A6).

TABLE 17
Incidence of Selected Nonneoplastic Lesions in Mice in the 14-Week Gavage Study of Acrolein

	Vehicle Control	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg
Male						
Forestomach[a]	10	10	10	10	10	10
Epithelium, Hyperplasia, Squamous[b]	0	2 (1.0)[c]	6** (1.0)	7** (1.1)	10** (2.0)	0
Glandular Stomach	10	10	10	9	10	10
Hemorrhage	0	0	0	0	1 (1.0)	10** (1.2)
Female						
Forestomach	10	10	10	9	10	10
Epithelium, Hyperplasia Squamous	0	0	4* (1.0)	7** (1.1)	8** (1.3)	2 (1.5)
Glandular Stomach	10	10	10	9	10	10
Epithelium, Necrosis	0	0	0	0	0	4* (3.3)
Hemorrhage	0	0	0	0	0	10** (1.8)
Inflammation, Chronic Active	0	0	0	0	0	5* (2.6)

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

GENETIC TOXICOLOGY

Allyl acetate was mutagenic in *Salmonella typhimurium* strains TA100 and TA1535 in the absence of S9 activation; no mutagenicity was detected in these strains with S9 (Table G1). Furthermore, negative results were obtained with allyl acetate in the *S. typhimurium* assay in strains TA97 and TA98, with and without S9. No significant increases in induction of micronucleated erythrocytes were noted in bone marrow samples from male rats administered allyl acetate by gavage three times at 24-hour intervals (Table G7). A small, but significant, increase in the frequency of micronucleated normochromatic erythrocytes (NCEs) was observed in peripheral blood of female mice administered allyl acetate by gavage for 14 weeks (Table G9). The mean value of NCEs in the 62.5 mg/kg group differed significantly (P=0.0006) from the vehicle control group, and the trend test was positive (P=0.001). No significant effect on micronucleus frequency was observed in male mice. For both male and female mice, the doses tested ranged from 8 to 62.5 mg/kg per day. Although data from two male mice treated with the high dose of 62.5 mg/kg are presented in Table G9, these data were not included in the statistical analysis because the experimental protocol requires a minimum of three surviving animals for a valid dose point. For males and females, the percentages of polychromatic erythrocytes in peripheral blood were unaffected by exposure to allyl acetate, indicating no alteration in cell cycling or turnover in the bone marrow.

Allyl alcohol was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA1535, with or without S9 metabolic activation (Table G2). No significant increases in induction of micronucleated erythrocytes were noted in bone marrow samples from male rats administered allyl alcohol by intraperitoneal injection three times at 24-hour intervals (Table G8). No significant increases in the frequencies of micronucleated normochromatic erythrocytes were observed in the peripheral blood of male or female mice administered allyl alcohol by gavage for 14 weeks (Table G10). The doses tested ranged from 3 to 50 mg/kg per day. There were no effects on the percentages of polychromatic erythrocytes among total erythrocytes for either gender, indicating no toxicity to the bone marrow resulting from exposure to allyl alcohol.

Acrolein showed evidence of mutagenicity in *S. typhimurium*, but little other indication of genetic activity was seen in the limited number of tests that were conducted. Acrolein was tested under two different protocols (preincubation and vapor) in the *S. typhimurium* gene mutation assay (Table G3). Testing under the preincubation protocol gave weakly positive results in strain TA100 in the presence of induced rat liver S9, and equivocal results in TA100 and TA1535 with induced hamster liver S9 (Haworth *et al.*, 1983). Negative results were obtained with strains TA98 and TA1537, with and without rat or hamster liver S9. Results of the *S. typhimurium* assay using the vapor protocol, where testing was carried out in the sealed environment of a desiccator to maximize exposure to this volatile chemical, were negative for all strains and activation conditions. The highest dose tested was 1 mL/chamber. Strains TA98 and TA1538 were also tested at this laboratory for mutagenicity to acrolein in a standard preincubation protocol; results were negative.

Acrolein induced a small but significant increase in sister chromatid exchanges in cultured Chinese hamster ovary cells in the absence of S9; this increase requires confirmation in a replicate trial (Table G4; Galloway *et al.*, 1987). No increase in sister chromatid exchanges was noted in the presence of S9 activation. Acrolein did not induce a significant increase in chromosomal aberrations in Chinese hamster ovary cells, with or without S9 (Table G5; Galloway *et al.*, 1987). No increases in the frequencies of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* were observed in three independent experiments with acrolein administered to adult flies via feeding or injection (Table G6; Zimmering *et al.*, 1985) and to larvae via feeding (Zimmering *et al.*, 1989).

In vivo, no increases in the frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood of male or female mice administered acrolein by gavage for 14 weeks (Table G11).

DISCUSSION

The metabolism of allyl acetate and other allyl esters involves initial hydrolysis of the ester linkage by carboxyl esterases to give allyl alcohol and, in the case of allyl acetate, acetic acid. Allyl alcohol is then oxidized to acrolein by alcohol dehydrogenase, and acrolein is oxidized to acrylic acid by aldehyde dehydrogenase. Because of the metabolic relationship of allyl acetate, allyl alcohol, and acrolein, and because acrolein is more toxic than the other two compounds, a comparative toxicity study was conducted to allow a simultaneous comparison of all three compounds in the same strains of rats and mice. Because several allyl esters are used as food additives and allyl acetate has been used as a flavoring agent in some countries, oral administration was selected as the route of exposure.

Dose selection was based on information available in the literature. The oral LD_{50} values for allyl acetate are 130 mg allyl acetate/kg body weight in rats and 170 mg/kg in mice (RTECS, 1991). Silver and Murphy (1978) examined the effect of carboxyl esterase inhibitors on the acute hepatotoxicity of allyl acetate in Holtzman rats; 90 mg/kg administered by gavage in corn oil was hepatotoxic but not lethal within the 24-hour time frame of their experiments, whereas 120 mg/kg caused mortality during the 24-hour experimental period. Based on the LD_{50} values and the results of Silver and Murphy (1978), doses of 0, 6, 12, 25, 50, and 100 mg/kg were selected for rats, and doses of 0, 8, 16, 32, 62.5, and 125 mg/kg were selected for mice, with 0.5% aqueous methyl cellulose as the vehicle.

The oral LD_{50} values for allyl alcohol are 64 mg/kg for rats and 85 to 96 mg/kg for mice (HSDB, 2001). The standard dose used in most hepatotoxicity studies is 50 mg/kg for rats and 60 mg/kg for mice; these doses are not lethal over the 24- to 48-hour duration of most published studies. Eigenberg *et al.* (1986) reported that oral doses of 50 or 75 mg/kg were hepatotoxic in F344 rats and $B6C3F_1$ mice and that mice administered 100 mg/kg died within 24 hours. Therefore, doses of 0, 1.5, 3, 6, 12, and 25 mg/kg in 0.5% aqueous methyl cellulose were selected for rats and doses of 0, 3, 6, 12, 25, and 50 mg/kg for mice.

Although numerous inhalation studies of acrolein have been published, data on oral exposure are limited. The oral LD_{50} values range from 26 to 30 mg/kg acrolein for rats (HSDB, 2001), although doses of 10 to 15 mg/kg may cause mortality with repeated administration. In a 2-year study in rats, Parent *et al.* (1992) reported that the high dose of 2.5 mg/kg produced no chemical-related lesions, although during 6-week prechronic studies, doses up to 15 mg/kg produced hepatotoxicity and some mortality in rats. Based on this information, doses of 0, 0.75, 1.25, 2.5, 5, and 10 mg/kg were selected for rats.

Mice appear less sensitive to allyl acetate and allyl alcohol toxicity than rats; therefore, the same was assumed true for acrolein. No oral LD_{50} value in mice is available for acrolein and limited data were available to guide dose selection. In a 2-year gavage study in mice conducted by Parent *et al.* (1991), the high dose of 4.5 mg/kg reduced survival but did not increase the incidences of microscopic lesions. Based on this information, doses of 0, 1.25, 2.5, 5, 10, and 20 mg/kg were selected for mice.

In the present studies, acrolein was clearly the most toxic of the three compounds in rats, causing reduced survival in the 2.5, 5, and 10 mg/kg groups and reduced body weights in the 10 mg/kg groups. Over the same dose range, allyl acetate administration caused only a marginal reduction in body weight gain, and allyl alcohol administration had no effect on survival or body weights of rats. Marginal increases in absolute or relative liver weights occurred in all three rat studies, mostly at the higher dose concentrations; however, no pattern of treatment-related changes was apparent in other organs.

Acrolein was also the most toxic of the three compounds in mice, causing reduced survival in the 20 mg/kg groups. Neither allyl alcohol nor allyl acetate caused reduced body weights or survival over the same dose range. Absolute and relative liver weights of male mice and relative liver weights of female mice were increased in the groups that received 10 mg/kg acrolein, and relative liver weights were increased in groups that received 50 mg/kg allyl alcohol.

The major toxic response for all three compounds occurred in the forestomach. Exposure to allyl alcohol was associated with only a mild response characterized by squamous epithelial hyperplasia. In rats, the severity was minimal in all dosed groups, but in mice the severity was mild in the 25 and 50 mg/kg allyl alcohol groups. A mild response also occurred in rats and mice administered 6 or 8 mg/kg allyl acetate, respectively; however, the incidences and severities of forestomach lesions increased with increasing dose. In the allyl acetate studies, epithelial necrosis, hemorrhage, and chronic active inflammation in the forestomach of 100 mg/kg rats and 62.5 mg/kg mice were thought to have contributed to the moribund condition of these animals. Epithelial hyperplasia of the forestomach was present in all groups of male mice exposed to acrolein and in females that received at least 2.5 mg/kg. Epithelial necrosis and/or hemorrhage also occurred in the glandular stomach of mice exposed to 20 mg/kg acrolein and contributed to the reduced survival in those groups.

Acrolein undergoes a Michael-type addition with glutathione, the sulfhydryl groups of proteins, and other sulfhydryl-containing compounds (Parent *et al.*, 1996a, 1998). The α-β double bond of the allyl group of acrolein is conjugated with electronegative carbonyl groups, thus enhancing acrolein's ability to undergo Michael addition. This is not true, however, for allyl acetate and allyl alcohol. Therefore, the toxic response in the forestomach that occurred in the allyl acetate and allyl alcohol studies may be the result of the metabolic conversion of these compounds to acrolein in the forestomach.

In the present studies, a toxic response also occurred in the liver of rats and mice administered allyl acetate and in mice and female rats administered allyl alcohol. Treatment with 25 mg/kg allyl alcohol, the highest dose evaluated, significantly increased the incidences of bile duct hyperplasia and periportal hepatocellular hypertrophy in female rats but not in males. In groups of mice administered allyl alcohol, females were somewhat more responsive than males, and increased incidences of portal cytoplasmic vacuolization occurred in 12 mg/kg or greater females; this lesion was first observed at 25 mg/kg in male mice.

Rikans and Moore (1987) reported a sex difference in allyl alcohol hepatotoxicity in rats that appeared to be correlated with the greater alcohol dehydrogenase activity in female rats than in male rats. As the male rats aged, the alcohol dehydrogenase activity in the liver increased, and their sensitivity to allyl alcohol hepatotoxicity also increased, although neither the alcohol dehydrogenase activity nor hepatotoxic response in older males became equal to that observed in young or old females.

In contrast to allyl alcohol, the hepatotoxic response to allyl acetate did not differ between males and females. Lesions similar to those that occurred in animals administered allyl alcohol also occurred in male and female rats administered 25 mg/kg allyl acetate; at higher doses the toxic response was more significant and included hepatocellular necrosis and other toxic lesions. A similar response occurred in male and female mice administered 62.5 mg/kg or greater. The increased alanine aminotransferase and sorbitol dehydrogenase activities in the serum of rats administered allyl acetate but not in rats administered allyl alcohol were consistent with the microscopic findings in the liver.

The periportal hepatotoxicity associated with allyl alcohol exposure is well documented (Badr, 1991), and, based on a number of observations, may be the result of the biotransformation of allyl alcohol to acrolein. However, acrolein administered at doses used in the present studies was not hepatotoxic in either rats or mice. After oral administration, acrolein is eliminated primarily in urine as a glutathione conjugate or oxidized to acrylic acid, which in turn is rapidly metabolized to carbon dioxide by the propionic acid pathway (Parent *et al.*, 1996a). As shown in Appendix F, 3-hydroxypropyl mercapturic acid, the major urinary metabolite of acrolein, was present in the urine of all groups of rats and mice exposed to allyl acetate or allyl alcohol, demonstrating the formation and detoxification of acrolein *in vivo* in these animals. However, Parent *et al.* (1996b) showed that acrolein also reacts with food in the intestinal tract. Therefore, although the local concentration of acrolein in the gut may have been sufficient to produce forestomach lesions, reaction with contents of the gastrointestinal tract must have reduced the systemic bioavailability to levels low enough to permit effective detoxification in the liver without causing a hepatotoxic response. Because neither allyl acetate nor allyl alcohol is as reactive as acrolein, their bioavailability would not have been reduced in the same way.

Horvath *et al.* (1992) reported that the 1:1 acrolein:glutathione adduct is activated to a nephrotoxin by γ-glutamyltranspeptidase; however, doses of 0.5 or 1 mM/kg were required for a toxic response. Although urinalysis demonstrated the presence of 3-hydroxypropyl mercapturate in the urine of all rats and mice exposed to allyl acetate,

allyl alcohol, or acrolein, no nephrotoxicity was observed in any groups, even though the highest dose groups of rats and mice that received allyl acetate or allyl alcohol received at least 1 mM/kg. This finding suggests that the steady-state concentration of the nephrotoxic adduct never reaches a toxic level.

Acrolein has been evaluated for carcinogenic potential in gavage studies in both rats and mice. In the rat study, doses 0, 0.05, 0.5, or 2.5 mg/kg were used (Parent *et al.*, 1992). In the present rat study, 2.5 mg/kg caused some alteration in clinical chemistry parameters and epithelial hyperplasia in the forestomach of males and females. Moreover, the incidences of forestomach hyperplasia increased substantially, especially in males, at the next highest dose concentration, 5 mg/kg. Therefore, the results of the present rat study indicate that the 2.5 mg/kg used in the Parent *et al.* (1992) rat study was an adequate high dose. In the present mouse study, the incidences of forestomach hyperplasia were significantly increased in 2.5 and 5 mg/kg males and females; therefore, the 4.5 mg/kg used in the Parent *et al.* (1991) study was an adequate high dose. Acrolein was not carcinogenic in the Parent *et al.* (1991, 1992) rat and mouse studies; therefore, it is quite likely that allyl alcohol and allyl acetate would also be negative in properly conducted carcinogenicity studies.

REFERENCES

Adams, J.D., Jr., and Klaidman, L.K. (1993). Acrolein-induced oxygen radical formation. *Free Radic. Biol. Med.* **15**, 187-193.

The Aldrich Library of FT-IR Spectra (1985). 1st ed. (C.J. Pouchert, Ed.), Vol. 1. Aldrich Chemical Company, Inc., Milwaukee, WI.

Arena, J.M., and Drew, R.H., Eds. (1986). *Poisoning-Toxicology, Symptoms, Treatments*. 5th ed., p. 275.

Badr, M.Z. (1991). Periportal hepatoxicity due to allyl alcohol: A myriad of proposed mechanisms. *J. Biochem. Toxicol.* **6**, 1-5.

Beauchamp, R.O., Jr., Andjelkovich, D.A., Kligerman, A.D., Morgan, K.T., and Heck, H.D. (1985). A critical review of the literature on acrolein toxicity. *Crit. Rev. Toxicol.* **14**, 309-380.

Belinsky, S.A., Bradford, B.U., Forman, D.T., Glassman, E.B., Felder, M.R., and Thurman, R.G. (1985). Hepatotoxicity due to allyl alcohol in deer mice depends on alcohol dehydrogenase. *Hepatology* **5**, 1179-1182.

Berhane, K., and Mannervik, B. (1990). Inactivation of the genotoxic aldehyde acrolein by human glutathione transferases of classes alpha, mu, and pi. *Mol. Pharmacol.* **37**, 251-254.

Bignami, M., Cardamone, G., Comba, P., Ortali, V.A., Morpurgo, G., and Carere, A. (1977). Relationship between chemical structure and mutagenic activity in some pesticides: The use of *Salmonella typhimurium* and *Aspirgillis nidulans*. *Mutat. Res.* **46**, 243-244.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Boorman, G.A., Hickman, R.L., Davis, G.W., Rhodes, L.S., White, N.W., Griffin, T.A., Mayo, J., and Hamm, T.E., Jr. (1986). Serological titers to murine viruses in 90-day and 2-year studies. In *Complications of Viral and Mycoplasmal Infections in Rodents to Toxicology Research and Testing* (T.E. Hamm, Jr., Ed.), pp. 11-23. Hemisphere Publishing Corporation, Washington, DC.

Boyd, J.W. (1983). The mechanisms relating to increases in plasma enzymes and isoenzymes in diseases of animals. *Vet. Clin. Pathol.* **12**, 9-24.

CEDRA Corporation (1996). Biological Sample Method Development for 3-Hydroxypropyl Mercapturic Acid in Rodent Urine. NIH Contract No. N01-ES-25332. CEDRA DCN A92-21-12/632.

Cannon, J., Linke, C.A., and Cos, L.R. (1991). Cyclophosphamide-associated carcinoma of urothelium: Modalities for prevention. *Urology* **38**, 413-416.

Code of Federal Regulations (CFR) **21**, Part 58.

Cohen, S.M., Garland, E.M., St. John, M., Okamura, T., and Smith, R.A. (1992). Acrolein initiates rat urinary bladder carcinogenesis. *Cancer Res.* **52**, 3577-3581.

Cooper, K.O., Witz, G., and Witmer, C. (1992). The effects of α,β-unsaturated aldehydes on hepatic thiols and thiol-containing enzymes. *Fundam. Appl. Toxicol.* **19**, 343-349.

Curren, R.D., Yang, L.L., Conklin, P.M., Grafstrom, R.C., and Harris, C.C. (1988). Mutagenesis of xeroderma pigmentosum fibroblasts by acrolein. *Mutat. Res.* **209**, 17-22.

Dean, B.J., Brooks, T.M., Hodson-Walker, G., and Hutson, D.H. (1985). Genetic toxicology testing of 41 industrial chemicals. *Mutat. Res.* **153**, 57-77.

Dixon, W.J., and Massey, F.J., Jr. (1957). *Introduction to Statistical Analysis*, 2nd. ed., pp. 276-278, 412. McGraw-Hill Book Company, Inc., New York.

Draminski, W., Eder, E., and Henschler, D. (1983). A new pathway of acrolein metabolism in rats. *Arch. Toxicol.* **52**, 243-247.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Eder, E., Hoffman, C., Sporer, S., and Scheckenbach, S. (1993). Biomonitoring studies and susceptibility markers for acrolein congeners and allylic and benzyl compounds. *Environ. Health Perspect.* **99**, 245-247.

Eigenberg, D.A., Carter, D.E., Schram, K.H., and Sipes, I.G. (1986). Examination of the differential hepatotoxicity of diallyl phthalate in rats and mice. *Toxicol. Appl. Pharmacol.* **86**, 12-21.

Finco, D.R. (1989). Kidney function. In *Clinical Biochemistry of Domestic Animals*, 4th ed. (J.J. Kaneko, Ed.), pp. 496-542. Academic Press, Inc., San Diego.

Foiles, P.G., Akerkar, S.A., and Chung, F.L. (1989). Application of an immunoassay for cyclic acrolein deoxyguanosine adducts to assess their formation in DNA of *Salmonella typhimurium* under conditions of mutation induction by acrolein. *Carcinogenesis* **10,** 87-90.

Foiles, P.G., Akerkar, S.A., Migleitta, L.M., and Chung, F.L. (1990). Formation of cyclic deoxyguanosine adducts in Chinese hamster ovary cells by acrolein and crotonaldehyde. *Carcinogenesis* **11**, 2059-2061.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* **10** (Suppl. 10), 1-175.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *JNCI* **62**, 957-974.

Hales, B.F. (1982). Comparison of the mutagenicity and the teratogenicity of cyclophosphamide and its active metabolites, 4-hydroxycyclophosphamide, phosphoramide mustard, and acrolein. *Cancer Res.* **42**, 3016-3021.

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., and Zeiger, E. (1983). Salmonella mutagenicity test results for 250 chemicals. *Environ. Mutagen.* **5** (Suppl. 1), 3-142.

Hazardous Substances Data Bank (HSDB) (2001). Maintained by the National Library of Medicine. Retrieved August 16, 2001, from the World Wide Web: <http://www.toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>.

Henschler, D., and Eder, E. (1986). Structure-activity relationships of alpha, beta-unsaturated carbonylic compounds. *IARC Sci. Publ.* **70**, 197-205.

Hofmann, A.F. (1988). Bile acids. In *The Liver: Biology and Patholobiology* (I.M. Arias, W.B. Jakoby, H. Popper, D. Schachter, and D.A. Shafritz, Eds.), pp. 553-572. Raven Press, Ltd., New York.

Hollander, M., and Wolfe, D.A. (1973). *Nonparametric Statistical Methods*, pp. 120-123. John Wiley and Sons, New York.

Horvath, J.J., Witmer, C.M., and Witz, G. (1992). Nephrotoxicity of the 1:1 acrolein-glutathione adduct in the rat. *Toxicol. Appl. Pharmacol.* **117**, 200-207.

Integrated Laboratory Systems (ILS) (1990). Micronucleus Data Management and Statistical Analysis Software, Version 1.4. ILS, P.O. Box 13501, Research Triangle Park, NC 27707.

Jain, N.C. (1986). Clinical and laboratory evaluation of anemias and polycythemias. In *Schalm's Veterinary Hematology*, 4th ed., pp. 563-576. Lea and Febiger, Philadelphia.

Jenkinson, P.C., and Anderson, D. (1990). Malformed foetuses and karyotype abnormalities in the offspring of cyclophosphamide and allyl alcohol-treated male rats. *Mutat. Res.* **229**, 173-184.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaye, C.M. (1973). Biosynthesis of mercapturic acids from allyl alcohol, allyl esters, and acrolein. *Biochem. J.* **134**, 1093-1101.

Kehrer, J.P., and Biswal, S.S. (2000). The molecular effects of acrolein. *Toxicol. Sci.* **57**, 6-15.

Lijinsky, W., and Reuber, M.D. (1987). Chronic carcinogenesis studies of acrolein and related compounds. *Toxicol. Ind. Health* **3**, 337-345.

Linhart, I., Frantik, E., Vodickova, L., Vosmanska, M., Smejkal, J., and Mitera, J. (1996). Biotransformation of acrolein in rat: Excretion of mercapturic acids after inhalation and intraperitoneal injection. *Toxicol. Appl. Pharmacol.* **136**, 155-160.

Lutz, D., Eder, E., Neudecker, T., and Henschler, D. (1982). Structure-mutagenicity relationship in α,β-unsaturated carbonylic compounds and their corresponding allylic alcohols. *Mutat. Res.* **93**, 305-315.

McDiarmid, M.A., Iype, P.T., Kolodner, K., Jacobson-Kram, D., and Strickland, P.T. (1991). Evidence for acrolein-modified DNA in peripheral blood leukocytes of cancer patients treated with cyclophosphamide. *Mutat. Res.* **248**, 93-99.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.S. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

Margolin, B.H., Collings, B.J., and Mason, J.M. (1983). Statistical analysis and sample-size determinations for mutagenicity experiments with binomial responses. *Environ. Mutagen.* **5**, 705-716.

Marnett, L.J., Hurd, H.K., Holstein, M.C., Levin, D.E., Esterbauer, H., and Ames, B.N. (1985). Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. *Mutat. Res.* **148**, 25-34.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Mason, J.M., Valencia, R., and Zimmering, S. (1992). Chemical mutagenesis testing in *Drosophila*: VIII. Reexamination of equivocal results. *Environ. Mol. Mutagen.* **19**, 227-234.

The Merck Index (1989). 11th ed. (S. Budavari, Ed.), p. 49. Merck and Company, Rahway, N.J.

Morrison, D.F. (1976). *Multivariate Statistical Methods*, 2nd ed., pp. 170-179. McGraw-Hill Book Company, New York.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., and Zeiger, E. (1986). *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutagen.* **8** (Suppl. 7), 1-119.

National Toxicology Program (NTP) (1991). Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluations in Toxicity Testing for Rats and Mice, 10/31/82 version (updated May 1991). Research Triangle Park, NC.

National Toxicology Program (NTP) (1993). Nomination history.

Nielsen, G.D., Bakbo, J.C., and Holst, E. (1984). Sensory irritation and pulmonary irritation by airborne allyl acetate, allyl alcohol, and allyl ether compared to acrolein. *Acta Pharmacol. Toxicol.* **54**, 292-298.

Pappas, N.J., Jr. (1989). Theoretical aspects of enzymes in diagnosis. Why do serum enzymes change in hepatic, myocardial, and other diseases? *Clin. Lab. Med.* **9**, 595-626.

Parent, R.A., Caravello, H.E., and Long, J.E. (1991). Oncogenicity study of acrolein in mice. *J. Am. Coll. Toxicol.* **10**, 647-659.

Parent, R.A., Caravello, H.E., and Long, J.E. (1992). Two-year toxicity and carcinogenicity study of acrolein in rats. *J. Appl. Toxicol.* **12**, 131-139.

Parent, R.A., Caravello, H.E., Christian, M.S., and Hoberman, A.M. (1993). Developmental toxicity of acrolein in New Zealand white rabbits. *Fundam. Appl. Toxicol.* **20**, 248-256.

Parent, R.A., Caravello, H.E., and Sharp, D.E. (1996a). Metabolism and distribution of [2,3-^{14}C]acrolein in Sprague-Dawley rats. *J. Appl. Toxicol.* **16**, 449-457.

Parent, R.A., Caravello, H.E., and San, R.H. (1996b). Mutagenic activity of acrolein in *S. typhimurium* and *E. coli*. *J. Appl. Toxicol.* **16**, 103-108.

Parent, R.A., Paust, D.E., Schrimpf, M.K., Talaat, R.E., Doane, R.A., Caravello, H.E., Lee, S.J., and Sharp, D.E. (1998). Metabolism and distribution of [2,3-^{14}C]acrolein in Sprague-Dawley rats. II. Identification of urinary and fecal metabolites. *Toxicol. Sci.* **43**, 110-120.

Rao, G.N., Haseman, J.K., and Edmondson, J. (1989a). Influence of viral infections on body weight, survival, and tumor prevalence in Fischer 344/NCr rats on two-year studies. *Lab. Anim. Sci.* **39**, 389-393.

Rao, G.N., Piegorsch, W.W., Crawford, D.D., Edmondson, J., and Haseman, J.K. (1989b). Influence of viral infections on body weight, survival, and tumor prevalence of B6C3F1 (C57BL/6N × C3H/HeN) mice in carcinogenicity studies. *Fundam. Appl. Toxicol.* **13**, 156-164.

Registry of Toxic Effects of Chemical Substances (RTECS) [database online] (1991). Bethesda (MD): National Institute for Occupational Safety and Health; 1971 to present. Updated quarterly. Available from the National Library of Medicine, Bethesda, MD.

Reid, W.D. (1972). Mechanism of allyl alcohol-induced hepatic necrosis. *Experientia* **28**, 1058-1061.

Rikans, L.E. (1987). The oxidation of acrolein by rat liver aldehyde dehydrogenases. Relation to allyl alcohol hepatotoxicity. *Drug Metab. Dispos.* **15**, 356-362.

Rikans, L.E., and Moore, D.R. (1987). Effect of age and sex on allyl alcohol hepatotoxicity in rats: Role of liver alcohol and aldehyde dehydrogenase activities. *J. Pharmacol. Exp. Ther.* **243**, 20-26.

Rosen, F., Roberts, N.R., Budnick, L.E., and Nichol, C.A. (1959a). Corticosteroids and transaminase activity: The specificity of the glutamic-pyruvic transaminase response. *Endocrinology* **65**, 256-264.

Rosen, F., Roberts, N.R., and Nichol, C.A. (1959b). Glucocorticosteroids and transaminase activity. I. Increased activity of glutamic-pyruvic transaminase in four conditions associated with gluconeogenesis. *J. Biol. Chem.* **234**, 476-480.

Rosen, J.D., Segall, Y., and Casida, J.E. (1980). Mutagenic potency of haloacroleins and related compounds. *Mutat. Res.* **78**, 113-119.

Sandmeyer, E.E., and Kirwin, C.J., Jr. (1989). Esters. In *Patty's Industrial Hygiene and Toxicology*, 3rd ed. (G.D. Clayton and F.E. Clayton, Eds.), Vol 2a, pp. 2259-2412. John Wiley and Sons, Inc., New York.

Sanduja, R., Ansari, G.A., and Boor, P.J. (1989). 3-Hydroxypropylmercapturic acid: A biologic marker of exposure to allylic and related compounds. *J. Appl. Toxicol.* **9**, 235-238.

Sax, N.I., and Lewis, R.J., Sr. (1987). *Hawley's Condensed Chemical Dictionary*, 11th ed. Van Nostrand Reinhold, Co., New York.

Sax, N.I., and Lewis, R.J., Sr. (1989). *Dangerous Properties of Industrial Materials*, 7th ed. Van Nostrand Reinhold, Co., New York.

Schmidt, E., and Schmidt, F.W. (1987). Enzyme release. *J. Clin. Chem. Clin. Biochem.* **25**, 525-540.

Schmidt, F.W., and Schmidt, E. (1989). Diagnostic application of mitochondrial enzymes and isoenzymes. *Clin. Chim. Acta* **185**, 253-263.

Shelby, M.D., Erexson, G.L., Hook, G.J., and Tice, R.R. (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environ. Mol. Mutagen.* **21**, 160-179.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

Sierra, L.M., Barros, A.R., Garcia, M., Ferreiro, J.A., and Comendador, M.A. (1991). Acrolein genotoxicity in *Drosophila melanogaster.* I. Somatic and germinal mutagenesis under proficient repair conditions. *Mutat. Res.* **260**, 247-256.

Silver, E.H., and Murphy, S.D. (1978). Effect of carboxylesterase inhibitors on the acute hepatotoxicity of esters of allyl alcohol. *Toxicol. Appl. Pharmacol.* **45**, 377-389.

Smith, J.E. (1989). Iron metabolism and its diseases. In *Clinical Biochemistry of Domestic Animals* (J.J. Kaneko, Ed.), pp. 256-273. Academic Press, Inc., San Diego, CA.

Smith, R.A., Cohen, S.M., and Lawson, T.A. (1990). Acrolein mutagenicity in the V79 assay. *Carcinogenesis* **11**, 497-498.

Travlos, G.S., Morris, R.W., Elwell, M.R., Duke, A., Rosenblum, S., and Thompson, M.B. (1996). Frequency and relationships of clinical chemistry and liver and kidney histopathology findings in 13-week toxicity studies in rats. *Toxicology* **107**, 17-29.

Vershueren, K. (1983). *Handbook of Environmental Data on Organic Chemicals*, 2nd ed. Van Nostrand Reinhold Co., New York.

Weast, R.C., Ed. (1989). *CRC Handbook of Chemistry and Physics*, 70th ed. CRC Press, Inc., Boca Raton, FL.

Weiss, G. (1986). *Hazardous Chemicals Data Book*, 2nd ed. Noyes Data Corporation, Park Ridge, NJ.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Williams, D.A. (1986). A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. *Biometrics* **42**, 183-186.

Wilson, V.L., Foiles, P.G., Chung, F.L., Povey, A.C., Frank, A.A., and Harris, C.C. (1991). Detection of acrolein and crotonaldehyde DNA adducts in cultured human cells and canine peripheral blood lymphocytes by ^{32}P-postlabeling and nucleotide chromatography. *Carcinogenesis* **12**, 1483-1490.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* **19** (Suppl. 21), 2-141.

Zimmering, S., Mason, J.M., Valencia, R., and Woodruff, R.C. (1985). Chemical mutagenesis testing in *Drosophila*. II. Results of 20 coded compounds tested for the National Toxicology Program. *Environ. Mol. Mutagen.* **7**, 87-100.

Zimmering, S., Mason, J.M., and Valencia, R. (1989). Chemical mutagenesis testing in *Drosophila*. VII. Results of 22 coded compounds tested in larval feeding experiments. *Environ. Mol. Mutagen.* **14**, 245-251.

APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund						2
Natural deaths						8
Survivors						
Terminal sacrifice	10	10	10	10	10	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, colon	(10)				(10)	(6)
Inflammation, chronic active						1 (17%)
Epithelium, necrosis						4 (67%)
Intestine large, rectum	(10)				(10)	(6)
Inflammation, chronic active						1 (17%)
Epithelium, hemorrhage						2 (33%)
Epithelium, necrosis						4 (67%)
Intestine large, cecum	(10)				(10)	(3)
Hemorrhage						1 (33%)
Inflammation, chronic active						1 (33%)
Epithelium, necrosis						1 (33%)
Intestine small, duodenum	(9)				(10)	(8)
Hemorrhage						4 (50%)
Epithelium, necrosis						7 (88%)
Intestine small, ileum	(10)				(10)	(4)
Epithelium, necrosis						1 (25%)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage					1 (10%)	9 (90%)
Hepatodiaphragmatic nodule		2 (20%)		2 (20%)		
Inflammation, chronic		5 (50%)	3 (30%)	3 (30%)	1 (10%)	
Mineralization				1 (10%)		10 (100%)
Mitotic alteration						3 (30%)
Pigmentation, hemosiderin				1 (10%)	6 (60%)	
Bile duct, hyperplasia				1 (10%)	9 (90%)	10 (100%)
Hepatocyte, necrosis					4 (40%)	10 (100%)
Hepatocyte, vacuolization cytoplasmic	3 (30%)	1 (10%)	6 (60%)	5 (50%)	6 (60%)	
Hepatocyte, periportal, degeneration, hydropic					2 (20%)	9 (90%)
Hepatocyte, periportal, hypertrophy				5 (50%)	8 (80%)	9 (90%)
Hepatocyte, periportal, mitotic alteration				1 (10%)	3 (30%)	4 (40%)
Portal, fibrosis					4 (40%)	6 (60%)
Portal, inflammation, granulomatous				1 (10%)	4 (40%)	7 (70%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage						9 (90%)
Inflammation, chronic						9 (90%)
Epithelium, hyperplasia, squamous		2 (20%)	6 (60%)	5 (50%)	10 (100%)	4 (40%)
Epithelium, necrosis						9 (90%)
Epithelium, ulcer						1 (10%)
Stomach, glandular	(10)				(10)	(3)
Hemorrhage						1 (33%)
Epithelium, necrosis					1 (10%)	

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

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Cardiovascular System						
Heart	(10)				(10)	(3)
Cardiomyopathy, chronic	10 (100%)				5 (50%)	
Endocrine System						
Pituitary gland	(10)				(10)	(4)
Pars distalis, cyst					1 (10%)	1 (25%)
Thyroid gland	(10)				(10)	(3)
Ultimobranchial cyst	1 (10%)					
General Body System						
None						
Genital System						
Preputial gland	(10)				(10)	(3)
Inflammation, chronic active					1 (10%)	
Prostate	(10)				(10)	(3)
Inflammation, chronic active					3 (30%)	
Seminal vesicle	(10)				(10)	(3)
Atrophy						2 (67%)
Testes	(10)				(10)	(3)
Seminiferous tubule, degeneration						3 (100%)
Hematopoietic System						
Bone marrow	(10)				(10)	(10)
Fibrosis						1 (10%)
Hyperplasia						10 (100%)
Lymph node						(5)
Mediastinal, depletion lymphoid						1 (20%)
Mediastinal, hemorrhage						5 (100%)
Mediastinal, necrosis						1 (20%)
Lymph node, mandibular	(10)				(10)	(9)
Depletion lymphoid						7 (78%)
Hemorrhage						1 (11%)
Lymph node, mesenteric	(10)				(10)	(9)
Depletion lymphoid						6 (67%)
Hemorrhage						5 (56%)
Spleen	(10)				(10)	(10)
Lymphoid follicle, depletion cellular						9 (90%)
Red pulp, hematopoietic cell proliferation						6 (60%)
Thymus	(9)				(10)	(10)
Hemorrhage						6 (60%)
Thymocyte, necrosis						9 (90%)
Integumentary System						
None						

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)				(10)	(4)
Inflammation, chronic active	2 (20%)				1 (10%)	
Nose	(10)				(10)	(4)
Olfactory epithelium, necrosis						1 (25%)
Special Senses System						
None						
Urinary System						
Kidney	(10)				(10)	(4)
Cortex, cyst						1 (25%)
Renal tubule, regeneration	5 (50%)				6 (60%)	

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Natural deaths						10
Survivors						
Terminal sacrifice	10	10	10	10	10	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, rectum	(10)				(10)	(2)
Parasite metazoan	1 (10%)					
Intestine small, duodenum	(10)				(10)	(5)
Hemorrhage						2 (40%)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Basophilic			1 (10%)			
Hemorrhage						10 (100%)
Hepatodiaphragmatic nodule	1 (10%)		1 (10%)		2 (20%)	
Inflammation, chronic	6 (60%)	7 (70%)	7 (70%)	4 (40%)	1 (10%)	
Mineralization						7 (70%)
Pigmentation, hemosiderin				6 (60%)	9 (90%)	
Bile duct, hyperplasia				1 (10%)	10 (100%)	4 (40%)
Hepatocyte, necrosis					2 (20%)	10 (100%)
Hepatocyte, periportal, degeneration, hydropic					7 (70%)	3 (30%)
Hepatocyte, periportal, hypertrophy				7 (70%)	10 (100%)	6 (60%)
Hepatocyte, periportal, mitotic alteration				2 (20%)	9 (90%)	1 (10%)
Hepatocyte, periportal, vacuolization cytoplasmic						1 (10%)
Portal, fibrosis					10 (100%)	2 (20%)
Portal, inflammation, granulomatous				3 (30%)	10 (100%)	3 (30%)
Pancreas	(10)				(10)	(3)
Acinus, atrophy	1 (10%)				1 (10%)	
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(9)
Hemorrhage						9 (100%)
Inflammation, chronic active						5 (56%)
Epithelium, hyperplasia, squamous		1 (10%)	3 (30%)	9 (90%)	7 (70%)	1 (11%)
Epithelium, necrosis						9 (100%)
Stomach, glandular	(10)				(10)	(4)
Hemorrhage						1 (25%)
Cardiovascular System						
Heart	(10)				(10)	(4)
Cardiomyopathy, chronic	2 (20%)					2 (50%)
Endocrine System						
Pituitary gland	(10)				(10)	(3)
Pars distalis, cyst	1 (10%)				1 (10%)	

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

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
General Body System						
None						
Genital System						
Ovary	(10)	(1)		(2)	(10)	(3)
Cyst	1 (10%)	1 (100%)		2 (100%)	1 (10%)	
Hematopoietic System						
Bone marrow	(10)				(10)	(10)
Hyperplasia						10 (100%)
Inflammation					1 (10%)	
Lymph node						(2)
Mediastinal, hemorrhage						2 (100%)
Lymph node, mandibular	(10)				(10)	(5)
Depletion lymphoid						4 (80%)
Lymph node, mesenteric	(10)				(10)	(7)
Depletion lymphoid						2 (29%)
Hemorrhage						6 (86%)
Spleen	(10)				(10)	(9)
Lymphoid follicle, depletion cellular						8 (89%)
Red pulp, hematopoietic cell proliferation						7 (78%)
Thymus	(10)				(10)	(8)
Hemorrhage						5 (63%)
Thymocyte, necrosis						8 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)			(10)	(3)
Inflammation, chronic active	2 (20%)				2 (20%)	
Special Senses System						
None						
Urinary System						
Kidney	(10)				(10)	(3)
Mineralization					2 (20%)	
Renal tubule, regeneration	1 (10%)					

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 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 large, colon	(10)					(10)
Parasite metazoan	1 (10%)					
Intestine large, rectum	(10)					(10)
Parasite metazoan	3 (30%)					1 (10%)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule				1 (10%)	1 (10%)	
Inflammation, chronic	6 (60%)	9 (90%)	9 (90%)	6 (60%)	7 (70%)	7 (70%)
Bile duct, hyperplasia						1 (10%)
Hepatocyte, periportal, hypertrophy						1 (10%)
Mesentery	(1)					
Accessory spleen	1 (100%)					
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia, squamous						1 (10%)
Epithelium, hyperplasia, squamous				5 (50%)	7 (70%)	6 (60%)
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy, chronic	9 (90%)					7 (70%)
Endocrine System						
None						
General Body System						
None						
Genital System						
Prostate	(10)					(10)
Inflammation, chronic	1 (10%)					
Testes	(10)					(10)
Germinal epithelium, atrophy	1 (10%)					
Hematopoietic System						
None						
Integumentary 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 Rats in the 14-Week Gavage Study of Allyl Alcohol

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)				(9)
Inflammation, chronic	1 (10%)					
Inflammation, chronic active	2 (20%)					1 (11%)
Special Senses System						
None						
Urinary System						
Kidney	(10)					(10)
Renal tubule, regeneration	7 (70%)					8 (80%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	9	10	10
Other				1		
Animals examined microscopically	10	10	10	9	10	10
Alimentary System						
Intestine large, colon	(10)					(10)
Parasite metazoan	2 (20%)					
Liver	(10)	(10)	(10)	(9)	(10)	(10)
Basophilic focus			1 (10%)			
Hepatodiaphragmatic nodule		3 (30%)	1 (10%)	3 (33%)	2 (20%)	
Inflammation, chronic	9 (90%)	7 (70%)	9 (90%)	7 (78%)	9 (90%)	6 (60%)
Bile duct, hyperplasia						8 (80%)
Hepatocyte, necrosis						1 (10%)
Hepatocyte, periportal, hypertrophy						8 (80%)
Mesentery					(1)	
Inflammation, granulomatous					1 (100%)	
Mineralization					1 (100%)	
Stomach, forestomach	(10)	(10)	(10)	(9)	(10)	(10)
Epithelium, hyperplasia, squamous			1 (10%)	4 (44%)	9 (90%)	8 (80%)
Epithelium, ulcer		1 (10%)				
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy, chronic	3 (30%)					2 (20%)
Endocrine System						
Pituitary gland	(10)					(10)
Pars distalis, cyst						1 (10%)
General Body System						
None						
Genital System						
Ovary	(10)		(2)			(10)
Cyst			2 (100%)			1 (10%)
Hematopoietic System						
None						
Integumentary 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 Rats in the 14-Week Gavage Study of Allyl Alcohol

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)		(1)	(2)	(10)
Inflammation, chronic active	2 (20%)					2 (20%)
Special Senses System						
None						
Urinary System						
None						

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Gavage Study of Acrolein[a]

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental death					1	
Moribund						2
Natural deaths				2	1	7
Survivors						
Terminal sacrifice	10	10	10	8	8	1
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, colon	(10)			(2)	(10)	(10)
Parasite metazoan	2 (20%)					
Intestine large, rectum	(10)			(2)	(10)	(10)
Parasite metazoan	1 (10%)					
Intestine large, cecum	(10)			(2)	(10)	(10)
Hemorrhage						1 (10%)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	1 (10%)			1 (10%)		
Inflammation, chronic						2 (20%)
Necrosis						1 (10%)
Hepatocyte, centrilobular, degeneration					1 (10%)	
Pancreas	(10)			(2)	(10)	(10)
Acinus, atrophy					1 (10%)	1 (10%)
Salivary glands	(10)			(2)	(10)	(8)
Cytoplasmic alteration	3 (30%)					1 (13%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage						3 (30%)
Inflammation, chronic active						3 (30%)
Epithelium, hyperplasia, squamous				3 (30%)	8 (80%)	9 (90%)
Epithelium, necrosis						3 (30%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage					3 (30%)	5 (50%)
Epithelium, necrosis						1 (10%)
Cardiovascular System						
Heart	(10)			(2)	(10)	(9)
Cardiomyopathy, chronic	8 (80%)			2 (100%)	9 (90%)	3 (33%)
Endocrine System						
Pituitary gland	(10)			(2)	(10)	(9)
Rathke's cleft, developmental malformation					1 (10%)	
General Body System						
None						

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

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Gavage Study of Acrolein

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Genital System						
Preputial gland	(9)			(2)	(10)	(10)
Inflammation, chronic active	1 (11%)				1 (10%)	
Prostate	(10)			(2)	(10)	(10)
Inflammation, chronic active	1 (10%)				1 (10%)	1 (10%)
Testes	(10)			(2)	(10)	(10)
Atrophy					1	(10%)
Hematopoietic System						
Bone marrow	(10)			(2)	(10)	(10)
Hyperplasia						4 (40%)
Lymph node, mandibular	(10)			(2)	(10)	(8)
Necrosis					2 (20%)	
Lymph node, mesenteric	(10)			(2)	(10)	(10)
Necrosis						1 (10%)
Spleen	(10)			(2)	(10)	(10)
Lymphoid follicle, depletion cellular						5 (50%)
Lymphoid follicle, necrosis				1 (50%)	2 (20%)	1 (10%)
Red pulp, hematopoietic cell proliferation						3 (30%)
Thymus	(10)			(2)	(10)	(9)
Thymocyte, atrophy						3 (33%)
Thymocyte, necrosis				1 (50%)	2 (20%)	4 (44%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)			(10)	(10)	(10)
Edema				2 (20%)		2 (20%)
Inflammation, chronic active	2 (20%)				5 (50%)	3 (30%)
Nose	(10)			(2)	(10)	(9)
Hemorrhage						1 (11%)
Inflammation, acute	1 (10%)				1 (10%)	6 (67%)
Special Senses System						
None						
Urinary System						
Kidney	(10)			(2)	(10)	(10)
Mineralization	1 (10%)				3 (30%)	1 (10%)
Renal tubule, regeneration	10 (100%)				5 (50%)	2 (20%)

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Gavage Study of Acrolein[a]

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental death					1	
Moribund				1		2
Natural deaths			1	1		6
Survivors						
Terminal sacrifice	10	10	9	8	9	2
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, rectum	(10)		(1)	(2)	(10)	(10)
Parasite metazoan	1 (10%)					
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule		1 (10%)			1 (10%)	1 (10%)
Inflammation, chronic	1 (10%)	1 (10%)	2 (20%)	3 (30%)		
Pancreas	(10)		(1)	(2)	(10)	(10)
Inflammation	1 (10%)					
Acinus, atrophy	2 (20%)					1 (10%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Foreign body						1 (10%)
Hemorrhage						1 10%)
Inflammation, chronic active						3 (30%)
Epithelium, hyperplasia, squamous			3 (30%)	5 (50%)	8 (80%)	10 (100%)
Epithelium, inflammation, chronic active						1 (10%)
Epithelium, necrosis						3 (30%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage						4 (40%)
Inflammation, chronic active						1 (10%)
Epithelium, necrosis						1 (10%)
Cardiovascular System						
Heart	(10)		(1)	(2)	(10)	(10)
Cardiomyopathy, chronic						1 (10%)
Inflammation, chronic active	1 (10%)					
Endocrine System						
None						
General Body System						
None						

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

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Gavage Study of Acrolein

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Genital System						
Clitoral gland	(10)		(1)	(2)	(10)	(10)
Inflammation, chronic active	1 (10%)				4 (40%)	2 (20%)
Ovary	(10)	(1)	(2)	(3)	(10)	(10)
Cyst	1 (10%)	1 (100%)	1 (50%)		1 (10%)	
Hematopoietic System						
Bone marrow	(10)		(1)	(2)	(10)	(10)
Hyperplasia						6 (60%)
Lymph node, mesenteric	(10)		(1)	(2)	(10)	(10)
Necrosis				1 (50%)		1 (10%)
Spleen	(10)		(1)	(2)	(10)	(10)
Lymphoid follicle, depletion cellular				1 (50%)		2 (20%)
Lymphoid follicle, necrosis						3 (30%)
Thymus	(10)		(1)	(2)	(10)	(9)
Thymocyte, atrophy						2 (22%)
Thymocyte, necrosis				1 (50%)		2 (22%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(4)	(3)	(4)	(10)	(10)
Edema				1 (25%)		2 (20%)
Inflammation, chronic active	4 (40%)			1 (25%)	3 (30%)	
Nose	(10)		(1)	(2)	(10)	(10)
Inflammation, acute				1 (50%)		5 (50%)
Special Senses System						
None						
Urinary System						
Kidney	(10)		(1)	(2)	(10)	(10)
Mineralization	4 (40%)		1 (100%)		3 (30%)	3 (30%)
Renal tubule, regeneration	1 (10%)					

APPENDIX B
SUMMARY OF NONNEOPLASTIC LESIONS IN MICE

TABLE B1
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	8 mg/kg	16 mg/kg	32 mg/kg	62.5 mg/kg	125 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental deaths		3		1	3	
Moribund						2
Natural deaths	1				5	8
Survivors						
Terminal sacrifice	9	7	10	9	2	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(3)		(10)	(10)	(3)
Inflammation, chronic active		1 (33%)				
Perforation		1 (33%)			3 (30%)	
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Fibrosis					5 (50%)	1 (10%)
Inflammation, chronic	2 (20%)	3 (30%)		1 (10%)	1 (10%)	2 (20%)
Mineralization					5 (50%)	1 (10%)
Necrosis		1 (10%)				
Pigmentation, hemosiderin					3 (30%)	
Bile duct, hyperplasia					2 (20%)	1 (10%)
Hepatocyte, necrosis					5 (50%)	10 (100%)
Hepatocyte, vacuolization cytoplasmic				1 (10%)	1 (10%)	
Portal, inflammation, granulomatous					2 (20%)	1 (10%)
Portal, vacuolization cytoplasmic					6 (60%)	
Pancreas	(10)	(3)		(10)	(10)	(3)
Cyst	1 (10%)					
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage					1 (10%)	5 (50%)
Inflammation, chronic active					1 (10%)	1 (10%)
Epithelium, hyperkeratosis		2 (20%)				
Epithelium, hyperplasia, squamous				4 (40%)	10 (100%)	1 (10%)
Epithelium, necrosis						1 (10%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage					4 (40%)	8 (80%)
Epithelium, necrosis						1 (10%)
Cardiovascular System						
Heart	(10)	(3)		(10)	(10)	(3)
Inflammation, chronic						1 (33%)
Mineralization					1 (10%)	1 (33%)
Epicardium, inflammation, chronic active		1 (33%)				
Endocrine System						
Adrenal cortex	(10)	(3)		(10)	(10)	(3)
Necrosis		1 (33%)				
Parathyroid gland	(5)			(7)	(5)	(2)
Cyst	1 (20%)					

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

TABLE B1
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	8 mg/kg	16 mg/kg	32 mg/kg	62.5 mg/kg	125 mg/kg
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Bone marrow	(10)	(3)		(10)	(10)	(3)
Myeloid cell, hyperplasia		1 (33%)				
Lymph node				(1)	(2)	
Mediastinal, inflammation					1 (50%)	
Mediastinal, necrosis				1 (100%)	1 (50%)	
Lymph node, mandibular	(9)	(2)		(9)	(10)	(5)
Necrosis				1 (11%)	5 (50%)	2 (40%)
Lymph node, mesenteric	(10)	(1)		(10)	(10)	(7)
Necrosis					5 (50%)	5 (71%)
Spleen	(10)	(3)		(10)	(10)	(8)
Hematopoietic cell proliferation		1 (33%)			1 (10%)	1 (13%)
Lymphoid follicle, necrosis		1 (33%)		1 (10%)	7 (70%)	7 (88%)
Thymus	(10)	(2)		(10)	(10)	(10)
Thymocyte, atrophy		1 (50%)				1 (10%)
Thymocyte, necrosis				1 (10%)	7 (70%)	9 (90%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(3)		(10)	(10)	(3)
Mediastinum, inflammation, chronic active		2 (67%)		1 (10%)	3 (30%)	
Special Senses System						
None						
Urinary System						
Kidney	(10)	(3)		(10)	(10)	(3)
Inflammation, chronic active				1 (10%)		
Renal tubule, regeneration				1 (10%)		

TABLE B2
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	8 mg/kg	16 mg/kg	32 mg/kg	62.5 mg/kg	125 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental deaths	1	2	4	2	4	
Moribund						2
Natural deaths				1		8
Survivors						
Terminal sacrifice	9	8	6	7	6	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(2)	(4)	(3)	(10)	(3)
Inflammation, chronic active				1 (33%)	1 (10%)	
Perforation		1 (50%)	3 (75%)	1 (33%)	2 (20%)	
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic	1 (10%)	3 (30%)	5 (50%)	2 (20%)	4 (40%)	1 (10%)
Mineralization						1 (10%)
Necrosis					2 (20%)	
Pigmentation, hemosiderin					2 (20%)	
Hepatocyte, necrosis						10 (100%)
Portal, vacuolization cytoplasmic				3 (30%)	4 (40%)	
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active						1 (10%)
Epithelium, hyperkeratosis				1 (10%)		
Epithelium, hyperplasia, squamous			4 (40%)	5 (50%)	8 (80%)	1 (10%)
Epithelium, necrosis						1 (10%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage						4 (40%)
Cardiovascular System						
Heart	(10)	(2)	(4)	(3)	(10)	(3)
Inflammation, chronic						1 (33%)
Mineralization						1 (33%)
Endocrine System						
Parathyroid gland	(5)	(1)	(4)	(2)	(7)	(2)
Cyst			1 (25%)			
General Body System						
None						
Genital System						
None						

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

TABLE B2
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	8 mg/kg	16 mg/kg	32 mg/kg	62.5 mg/kg	125 mg/kg
Hematopoietic System						
Bone marrow	(10)	(2)	(4)	(3)	(10)	(3)
Myeloid cell, hyperplasia						1 (33%)
Lymph node			(1)	(1)	(1)	
Mediastinal, necrosis			1 (100%)	1 (100%)	1 (100%)	
Lymph node, mandibular	(9)	(1)	(3)	(3)	(8)	(5)
Necrosis			2 (67%)		1 (13%)	4 (80%)
Lymph node, mesenteric	(10)	(2)	(4)	(3)	(10)	(6)
Necrosis		1 (50%)	1 (25%)	1 (33%)	4 (40%)	5 (83%)
Spleen	(10)	(2)	(4)	(3)	(10)	(9)
Hematopoietic cell proliferation						1 (11%)
Lymphoid follicle, depletion cellular			1 (25%)			
Lymphoid follicle, necrosis	1 (10%)	2 (100%)	3 (75%)	2 (67%)	4 (40%)	8 (89%)
Thymus	(10)	(2)	(4)	(3)	(10)	(10)
Thymocyte, atrophy			2 (50%)			1 (10%)
Thymocyte, necrosis	1 (10%)	2 (100%)	2 (50%)	2 (67%)	4 (40%)	9 (90%)
Integumentary System						
Skin	(10)	(2)	(4)	(3)	(10)	(3)
Subcutaneous tissue, necrosis					1 (10%)	
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(2)	(4)	(3)	(10)	(3)
Inflammation, chronic active		1 (50%)				
Necrosis				1 (33%)		
Mediastinum, hemorrhage			1 (25%)			
Mediastinum, inflammation, chronic active	1 (10%)	2 (100%)	4 (100%)	2 (67%)	4 (40%)	
Nose	(10)	(2)	(4)	(3)	(10)	(3)
Inflammation, chronic active			1 (25%)			
Special Senses System						
None						
Urinary System						
None						

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg	50 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)	(10)	(10)	(10)	(10)
Inflammation						1 (10%)
Inflammation, chronic	7 (70%)	4 (40%)	4 (40%)	8 (80%)	7 (70%)	7 (70%)
Mineralization						1 (10%)
Pigmentation, hemosiderin						1 (10%)
Portal, vacuolization cytoplasmic					2 (20%)	10 (100%
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, hyperplasia, squamous		1 (10%)	3 (30%)	9 (90%)	10 (100%)	10 (100%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Cyst					1 (10%)	1 (10%)
Inflammation, chronic active	1 (10%)				1 (10%)	
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Testes	(10)					(10)
Mineralization						2 (20%)
Germinal epithelium, degeneration						1 (10%)
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						

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

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Gavage Study of Allyl Alcohol

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
None						

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental death						1
Survivors						
Terminal sacrifice	10	10	10	10	10	9
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic	10 (100%)	8 (80%)	8 (80%)	8 (80%)	9 (90%)	9 (90%)
Inflammation, granulomatous						1 (10%)
Pigmentation, hemosiderin						1 (10%)
Hepatocyte, necrosis						1 (10%)
Portal, vacuolization cytoplasmic	1 (10%)	1 (10%)	1 (10%)	5 (50%)	8 (80%)	9 (90%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, hyperplasia, squamous				8 (80%)	10 (100%)	9 (90%)
Stomach, glandular	(10)	(10)	(10)	(10)	(9)	(10)
Cyst						1 (10%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Spleen	(10)					(10)
Lymphoid follicle, necrosis						1 (10%)
Thymus	(10)					(10)
Thymocyte, necrosis						1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Gavage Study of Allyl Alcohol

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
None						

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Gavage Study of Acrolein[a]

	Vehicle Control	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental deaths		1		1		
Natural deaths					1	10
Survivors						
Terminal sacrifice	10	9	10	9	9	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(1)		(1)	(10)	(10)
Hemorrhage				1 (100%)		
Perforation		1 (100%)		1 (100%)		
Periesophageal tissue, inflammation, chronic active		1 (100%)		1 (100%)		
Intestine small, jejunum	(10)	(1)		(1)	(10)	(10)
Peyer's patch, necrosis						1 (10%)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation				3 (30%)		
Inflammation, chronic	6 (60%)	2 (20%)	1 (10%)	1 (10%)	3 (30%)	1 (10%)
Pancreas	(10)	(1)		(1)	(10)	(10)
Acinus, vacuolization cytoplasmic	1 (10%)					
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage						1 (10%)
Inflammation, chronic active				1 (10%)	1 (10%)	
Epithelium, hyperplasia, squamous		2 (20%)	6 (60%)	7 (70%)	10 (100%)	
Epithelium, necrosis					1 (10%)	
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage					1 (10%)	10 (100%)
Inflammation, acute	1 (10%)					
Epithelium, necrosis					1 (10%)	2 (20%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						

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

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Gavage Study of Acrolein

	Vehicle Control	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg
Hematopoietic System						
Bone marrow	(10)	(1)		(10)	(10)	(10)
Hyperplasia					1 (10%)	
Lymph node, mandibular	(9)			(1)	(9)	(9)
Necrosis						5 (56%)
Lymph node, mesenteric	(10)	(1)		(1)	(9)	(10)
Necrosis					1 (11%)	7 (70%)
Spleen	(10)	(1)		(1)	(10)	(10)
Lymphoid follicle, depletion cellular		1 (100%)			1 (10%)	10 (100%)
Thymus	(9)	(1)		(1)	(10)	(10)
Thymocyte, atrophy				1 (100%)		
Thymocyte, necrosis		1 (100%)			1 (10%)	10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)		(1)	(10)	(10)
Hemorrhage				1 (100%)		
Nose	(10)	(1)		(1)	(10)	(10)
Inflammation, acute					1 (10%)	
Special Senses System						
None						
Urinary System						
Kidney	(10)	(1)		(1)	(10)	(10)
Mineralization	1 (10%)				1 (10%)	
Renal tubule, regeneration	3 (30%)	1 (100%)				1 (10%)

TABLE B6
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Gavage Study of Acrolein[a]

	Vehicle Control	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental deaths	1				1	
Natural deaths					1	10
Survivors						
Terminal sacrifice	9	10	10	9	8	
Missing				1		
Animals Examined Microscopically	10	10	10	9	10	10
Alimentary System						
Esophagus	(10)				(10)	(10)
Hemorrhage					1 (10%)	
Inflammation, chronic active					1 (10%)	
Perforation					1 (10%)	
Periesophageal tissue, inflammation, chronic active					1 (10%)	
Liver	(10)	(10)	(10)	(9)	(10)	(10)
Inflammation, chronic	8 (80%)	2 (20%)	7 (70%)	3 (33%)	10 (100%)	1 (10%)
Stomach, forestomach	(10)	(10)	(10)	(9)	(10)	(10)
Hemorrhage						3 (30%)
Inflammation, chronic active						1 (10%)
Epithelium, hyperplasia, squamous			4 (40%)	7 (78%)	8 (80%)	2 (20%)
Epithelium, necrosis						1 (10%)
Stomach, glandular	(10)	(10)	(10)	(9)	(10)	(10)
Hemorrhage						10 (100%)
Inflammation, chronic active						5 (50%)
Epithelium, necrosis						4 (40%)
Cardiovascular System						
Heart	(10)				(10)	(10)
Degeneration						1 (10%)
Mineralization						4 (40%)
Endocrine System						
None						
General Body System						
None						
Genital System						
None						

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

TABLE B6
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Gavage Study of Acrolein

	Vehicle Control	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg
Hematopoietic System						
Bone marrow	(10)				(10)	(10)
Hyperplasia						2 (20%)
Lymph node, mandibular	(10)				(10)	(10)
Atrophy					1	(10%)
Necrosis					1 (10%)	5 (50%)
Lymph node, mesenteric	(10)				(10)	(9)
Atrophy					1	(11%)
Necrosis					2 (20%)	5 (56%)
Spleen	(10)				(10)	(10)
Lymphoid follicle, atrophy						1 (10%)
Lymphoid follicle, depletion cellular					1 (10%)	8 (80%)
Thymus	(10)				(10)	(10)
Mineralization						1 (10%)
Thymocyte, atrophy						2 (20%)
Thymocyte, necrosis					2 (20%)	8 (80%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)				(10)	(10)
Hemorrhage					1 (10%)	
Inflammation, chronic active	1 (10%)					
Nose	(10)				(10)	(10)
Inflammation, acute					1 (10%)	
Trachea	(10)				(10)	(10)
Glands, cyst	1 (10%)					
Special Senses System						
None						
Urinary System						
Kidney	(10)				(10)	(10)
Renal tubule, regeneration	1 (10%)					1 (10%)

APPENDIX C
CLINICAL PATHOLOGY RESULTS

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	10	9	2
Day 23	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Hematocrit (%)						
Day 4	42.4 ± 0.7	41.9 ± 0.5	41.8 ± 0.4	40.7 ± 0.4	41.2 ± 0.6	35.7 ± 3.8
Day 23	46.1 ± 0.4	46.1 ± 0.5	45.9 ± 0.4	46.2 ± 0.5	44.5 ± 0.4	
Week 14	46.2 ± 0.6	46.4 ± 0.4	46.1 ± 0.4	45.9 ± 0.4	45.7 ± 0.5	
Hemoglobin (g/dL)						
Day 4	13.7 ± 0.2	13.5 ± 0.1	13.6 ± 0.2	13.2 ± 0.1	13.4 ± 0.2	11.9 ± 1.3
Day 23	15.3 ± 0.2	15.4 ± 0.1	15.2 ± 0.1	15.2 ± 0.2	14.9 ± 0.2	
Week 14	15.0 ± 0.2	15.2 ± 0.1	15.1 ± 0.1	15.0 ± 0.1	14.9 ± 0.1	
Erythrocytes (10^6/μL)						
Day 4	7.04 ± 0.14	7.04 ± 0.09	7.04 ± 0.08	6.81 ± 0.06	7.03 ± 0.10	6.16 ± 0.56
Day 23	7.61 ± 0.09	7.67 ± 0.10	7.65 ± 0.06	7.66 ± 0.09	7.58 ± 0.09	
Week 14	8.50 ± 0.13	8.62 ± 0.07	8.58 ± 0.07	8.51 ± 0.06	8.71 ± 0.09	
Reticulocytes (10^6/μL)						
Day 4	0.39 ± 0.02	0.42 ± 0.01	0.38 ± 0.02	0.41 ± 0.02	0.42 ± 0.02	0.61 ± 0.14
Day 23	0.22 ± 0.01	0.19 ± 0.02	0.20 ± 0.01	0.19 ± 0.01	0.22 ± 0.02	
Week 14	0.15 ± 0.02	0.13 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	0.16 ± 0.02	
Nucleated erythrocytes (10^3/μL)						
Day 4	0.03 ± 0.02	0.02 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	0.07 ± 0.03	1.50 ± 1.50
Day 23	0.06 ± 0.03	0.00 ± 0.00	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
Mean cell volume (fL)						
Day 4	60.4 ± 0.5	59.5 ± 0.2	59.5 ± 0.3	59.6 ± 0.5	58.8 ± 0.1**	58.0 ± 1.0*
Day 23	60.6 ± 0.5	60.2 ± 0.3	60.1 ± 0.3	60.4 ± 0.4	58.9 ± 0.5	
Week 14	54.4 ± 0.3	53.7 ± 0.2	53.9 ± 0.1	54.0 ± 0.2	52.5 ± 0.2**	
Mean cell hemoglobin (pg)						
Day 4	19.5 ± 0.1	19.2 ± 0.1	19.4 ± 0.1	19.3 ± 0.2	19.1 ± 0.1	19.3 ± 0.4
Day 23	20.2 ± 0.1	20.1 ± 0.2	19.8 ± 0.1*	19.9 ± 0.1	19.6 ± 0.1**	
Week 14	17.7 ± 0.1	17.6 ± 0.1	17.6 ± 0.1	17.6 ± 0.1	17.1 ± 0.1**	
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.3 ± 0.2	32.3 ± 0.2	32.6 ± 0.1	32.4 ± 0.4	32.5 ± 0.1	33.4 ± 0.2
Day 23	33.2 ± 0.1	33.4 ± 0.2	33.1 ± 0.2	33.0 ± 0.2	33.4 ± 0.3	
Week 14	32.5 ± 0.1	32.7 ± 0.1	32.7 ± 0.1	32.7 ± 0.2	32.6 ± 0.1	
Platelets (10^3/μL)						
Day 4	777.5 ± 34.3	905.7 ± 20.5*	893.3 ± 33.7*	863.2 ± 32.4	726.9 ± 49.1	179.5 ± 99.5
Day 23	779.3 ± 18.3	752.6 ± 12.0	742.4 ± 16.0	721.3 ± 17.0	831.9 ± 26.6	
Week 14	641.3 ± 10.1	650.4 ± 14.1	663.7 ± 10.9	696.0 ± 14.7*	804.9 ± 23.6**	
Leukocytes (10^3/μL)						
Day 4	8.99 ± 0.53	9.92 ± 0.68	9.69 ± 0.38	8.82 ± 0.52	9.69 ± 0.74	8.95 ± 4.15
Day 23	10.86 ± 0.67	9.45 ± 0.39	10.15 ± 0.34	9.19 ± 0.50*	8.68 ± 0.51**	
Week 14	7.14 ± 0.39	7.72 ± 0.42	8.08 ± 0.24	7.47 ± 0.39	8.16 ± 0.39	
Segmented neutrophils (10^3/μL)						
Day 4	1.19 ± 0.15	1.42 ± 0.17	1.64 ± 0.12	1.56 ± 0.14	1.97 ± 0.26*	1.53 ± 0.57
Day 23	1.12 ± 0.11	1.23 ± 0.10	1.11 ± 0.11	1.18 ± 0.10	1.30 ± 0.15	
Week 14	1.19 ± 0.11	1.39 ± 0.08	1.49 ± 0.13	1.21 ± 0.10	1.71 ± 0.13**	

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	10	9	2
Day 23	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Lymphocytes (10^3/μL)						
Day 4	7.61 ± 0.51	8.30 ± 0.62	7.88 ± 0.39	7.06 ± 0.49	7.54 ± 0.59	6.67 ± 3.02
Day 23	9.54 ± 0.57	8.13 ± 0.37	8.93 ± 0.36	7.90 ± 0.48*	7.26 ± 0.38**	
Week 14	5.66 ± 0.34	6.03 ± 0.37	6.22 ± 0.21	6.05 ± 0.34	6.19 ± 0.34	
Monocytes (10^3/μL)						
Day 4	0.13 ± 0.03	0.14 ± 0.04	0.13 ± 0.03	0.14 ± 0.03	0.16 ± 0.06	0.42 ± 0.23
Day 23	0.12 ± 0.04	0.06 ± 0.02	0.06 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	
Week 14	0.17 ± 0.04	0.20 ± 0.04	0.26 ± 0.03	0.11 ± 0.03	0.17 ± 0.04	
Eosinophils (10^3/μL)						
Day 4	0.05 ± 0.02	0.06 ± 0.03	0.05 ± 0.02	0.06 ± 0.03	0.02 ± 0.01	0.33 ± 0.33
Day 23	0.08 ± 0.03	0.03 ± 0.02	0.05 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	
Week 14	0.13 ± 0.03	0.11 ± 0.02	0.11 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	2
Day 23	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Urea nitrogen (mg/dL)						
Day 4	11.1 ± 0.5	11.5 ± 0.4	11.6 ± 0.4	10.0 ± 0.7	8.2 ± 0.5**	23.0 ± 17.0
Day 23	11.3 ± 0.4	12.0 ± 0.3	11.3 ± 0.4	12.1 ± 0.8	11.2 ± 0.3	
Week 14	16.2 ± 0.6	14.3 ± 0.4*	15.5 ± 0.3	15.7 ± 0.5	15.4 ± 0.4	
Creatinine (mg/dL)						
Day 4	0.57 ± 0.02[b]	0.60 ± 0.00	0.59 ± 0.01	0.57 ± 0.02	0.56 ± 0.02	0.50 ± 0.00
Day 23	0.66 ± 0.02	0.71 ± 0.05	0.66 ± 0.02	0.66 ± 0.02	0.63 ± 0.02	
Week 14	0.77 ± 0.02	0.70 ± 0.00**	0.74 ± 0.02	0.71 ± 0.01*	0.71 ± 0.01*	
Total protein (g/dL)						
Day 4	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.4 ± 0.1	5.5 ± 0.1	4.5 ± 0.5*
Day 23	6.0 ± 0.1	6.1 ± 0.1	5.9 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	
Week 14	6.5 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.5 ± 0.0	6.6 ± 0.1	
Albumin (g/dL)						
Day 4	4.3 ± 0.1	4.2 ± 0.0	4.2 ± 0.1	4.1 ± 0.1*	3.8 ± 0.1**	2.8 ± 0.5**
Day 23	4.5 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	
Week 14	4.8 ± 0.1	4.6 ± 0.0	4.7 ± 0.0	4.7 ± 0.0	4.5 ± 0.1**	
Alanine aminotransferase (IU/L)						
Day 4	68 ± 2	70 ± 2	68 ± 2	73 ± 3	99 ± 13	625 ± 205**
Day 23	64 ± 1	66 ± 2	65 ± 2	61 ± 2	141 ± 31*	
Week 14	88 ± 3	83 ± 3	95 ± 4	99 ± 5	112 ± 16	
Alkaline phosphatase (IU/L)						
Day 4	1,404 ± 146	1,584 ± 102	1,463 ± 87	1,583 ± 29	1,200 ± 106**	1,271 ± 47
Day 23	1,193 ± 30	1,137 ± 14	1,147 ± 17	1,114 ± 23	1,161 ± 20	
Week 14	626 ± 16	588 ± 9	597 ± 7	583 ± 11*	552 ± 18**	

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	2
Day 23	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Creatine kinase (IU/L)						
Day 4	330 ± 30	378 ± 33	385 ± 42	430 ± 83	490 ± 91	1,271 ± 47*
Day 23	415 ± 76	334 ± 43	502 ± 84	331 ± 29	359 ± 64	
Week 14	228 ± 22	220 ± 33	278 ± 40	250 ± 41	338 ± 53	
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 2	14 ± 1	15 ± 1	15 ± 1	31 ± 6	217 ± 117
Day 23	24 ± 1	25 ± 2	22 ± 1	25 ± 2	53 ± 13**	
Week 14	29 ± 1	25 ± 2	28 ± 2	35 ± 3	39 ± 6	
Bile acids (μmol/L)						
Day 4	25.8 ± 2.6	20.9 ± 1.6	25.4 ± 2.5	30.9 ± 2.6	39.8 ± 6.3*	307.5 ± 25.5*
Day 23	15.0 ± 1.6	15.7 ± 1.0	17.0 ± 1.5	20.7 ± 2.2*	45.8 ± 8.3**	
Week 14	18.0 ± 1.0	21.8 ± 2.0	19.8 ± 2.7	21.2 ± 2.2	23.4 ± 2.3	
Female						
Hematology						
n						
Day 4	10	9	9	10	10	4
Day 23	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Hematocrit (%)						
Day 4	44.3 ± 0.5	45.7 ± 0.8	43.8 ± 0.7	43.5 ± 0.6	44.3 ± 0.6	25.2 ± 4.1*
Day 23	46.0 ± 0.5	46.1 ± 0.3	45.6 ± 0.5	46.2 ± 0.3	44.7 ± 0.5	
Week 14	44.5 ± 0.4	44.6 ± 0.6	43.9 ± 0.5	45.2 ± 0.4	43.0 ± 0.5	
Hemoglobin (g/dL)						
Day 4	14.2 ± 0.2	14.3 ± 0.3	13.8 ± 0.2	13.9 ± 0.2	14.1 ± 0.2	8.8 ± 1.3**
Day 23	15.2 ± 0.2	15.1 ± 0.1	15.0 ± 0.2	15.0 ± 0.1	14.6 ± 0.1**	
Week 14	14.7 ± 0.2	14.6 ± 0.2	14.5 ± 0.1	14.7 ± 0.1	14.0 ± 0.2*	
Erythrocytes (10^6/μL)						
Day 4	7.26 ± 0.09	7.39 ± 0.13	7.16 ± 0.11	7.06 ± 0.08	7.27 ± 0.08	3.84 ± 0.69*
Day 23	7.43 ± 0.08	7.45 ± 0.07	7.31 ± 0.09	7.39 ± 0.05	7.45 ± 0.09	
Week 14	7.63 ± 0.06	7.66 ± 0.10	7.52 ± 0.08	7.77 ± 0.08	7.69 ± 0.08	
Reticulocytes (10^6/μL)						
Day 4	0.26 ± 0.01	0.28 ± 0.02	0.27 ± 0.02	0.29 ± 0.02	0.28 ± 0.03	0.63 ± 0.05**
Day 23	0.13 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	
Week 14	0.09 ± 0.01	0.12 ± 0.01	0.12 ± 0.02	0.10 ± 0.01	0.12 ± 0.01	
Nucleated erythrocytes (10^3/μL)						
Day 4	0.06 ± 0.02	0.07 ± 0.03	0.02 ± 0.01	0.06 ± 0.02	0.08 ± 0.05	15.58 ± 7.13*
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	
Week 14	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	9	9	10	10	4
Day 23	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Mean cell volume (fL)						
Day 4	61.2 ± 0.3	61.8 ± 0.3	61.1 ± 0.2	61.7 ± 0.2	61.0 ± 0.4	66.0 ± 1.4**
Day 23	62.1 ± 0.3	62.1 ± 0.2	62.4 ± 0.2	62.6 ± 0.3	60.1 ± 0.4**	
Week 14	58.4 ± 0.2	58.3 ± 0.2	58.3 ± 0.2	58.2 ± 0.2	55.9 ± 0.2**	
Mean cell hemoglobin (pg)						
Day 4	19.5 ± 0.1	19.3 ± 0.1	19.3 ± 0.2	19.7 ± 0.2	19.3 ± 0.1	23.5 ± 0.9*
Day 23	20.5 ± 0.2	20.3 ± 0.1	20.5 ± 0.1	20.3 ± 0.1	19.6 ± 0.2**	
Week 14	19.3 ± 0.2	19.1 ± 0.1	19.3 ± 0.2	18.9 ± 0.1*	18.1 ± 0.1**	
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.0 ± 0.2	31.3 ± 0.2	31.6 ± 0.3	31.9 ± 0.3	31.7 ± 0.2	35.4 ± 0.7
Day 23	33.1 ± 0.3	32.8 ± 0.2	32.9 ± 0.1	32.5 ± 0.1	32.7 ± 0.3	
Week 14	33.1 ± 0.3	32.8 ± 0.2	33.0 ± 0.3	32.4 ± 0.1	32.4 ± 0.1	
Platelets (10^3/μL)						
Day 4	954.9 ± 15.4	920.3 ± 31.2	941.7 ± 32.3	952.6 ± 22.3	711.3 ± 47.7**	82.3 ± 14.6**
Day 23	702.1 ± 18.6	713.6 ± 15.6	748.2 ± 13.9	731.4 ± 13.4	975.0 ± 30.3**	
Week 14	675.5 ± 9.6	662.4 ± 10.7	664.6 ± 6.5	686.8 ± 13.1	809.2 ± 40.6*	
Leukocytes (10^3/μL)						
Day 4	9.46 ± 0.50	10.02 ± 0.76	9.28 ± 0.57	8.87 ± 0.88	11.08 ± 0.56	10.78 ± 2.05
Day 23	8.97 ± 0.32	8.94 ± 0.73	8.67 ± 0.67	8.78 ± 0.57	9.42 ± 0.75	
Week 14	6.34 ± 0.41	5.51 ± 0.30	5.91 ± 0.30	6.48 ± 0.31	7.57 ± 0.30*	
Segmented neutrophils (10^3/μL)						
Day 4	1.47 ± 0.17	1.36 ± 0.14	1.25 ± 0.14	1.27 ± 0.13	2.09 ± 0.15	2.64 ± 0.96
Day 23	1.15 ± 0.10	1.15 ± 0.14	1.11 ± 0.11	1.09 ± 0.10	1.38 ± 0.15	
Week 14	1.26 ± 0.09	1.20 ± 0.11	1.08 ± 0.10	1.06 ± 0.08	1.32 ± 0.06	
Lymphocytes (10^3/μL)						
Day 4	7.84 ± 0.46	8.45 ± 0.64	7.84 ± 0.49	7.46 ± 0.76	8.65 ± 0.54	7.98 ± 1.29
Day 23	7.55 ± 0.27	7.50 ± 0.58	7.28 ± 0.61	7.39 ± 0.57	7.56 ± 0.63	
Week 14	4.92 ± 0.38	4.15 ± 0.25	4.64 ± 0.27	5.21 ± 0.31	6.02 ± 0.27*	
Monocytes (10^3/μL)						
Day 4	0.09 ± 0.03	0.16 ± 0.06	0.11 ± 0.04	0.06 ± 0.03	0.28 ± 0.06*	0.11 ± 0.02
Day 23	0.21 ± 0.06	0.20 ± 0.04	0.17 ± 0.04	0.25 ± 0.05	0.32 ± 0.06	
Week 14	0.12 ± 0.03	0.11 ± 0.03	0.12 ± 0.03	0.12 ± 0.02	0.16 ± 0.03	
Eosinophils (10^3/μL)						
Day 4	0.06 ± 0.02	0.05 ± 0.04	0.08 ± 0.03	0.08 ± 0.03	0.06 ± 0.02	0.05 ± 0.03
Day 23	0.06 ± 0.02	0.09 ± 0.03	0.11 ± 0.03	0.05 ± 0.02	0.15 ± 0.03	
Week 14	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.08 ± 0.02	

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Allyl Acetate

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Female (continued)						
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	4
Day 23	10	10	10	9	10	0
Week 14	10	10	10	10	10	0
Urea nitrogen (mg/dL)						
Day 4	11.3 ± 0.5	10.3 ± 0.3	10.6 ± 0.5	10.9 ± 0.4	9.0 ± 0.6	29.5 ± 11.4
Day 23	13.3 ± 0.4	13.2 ± 0.3	13.7 ± 0.2	13.4 ± 0.4	14.3 ± 0.4	
Week 14	14.8 ± 0.5	15.0 ± 0.5	14.4 ± 0.5	14.6 ± 0.4	13.6 ± 0.3	
Creatinine (mg/dL)						
Day 4	0.61 ± 0.01	0.60 ± 0.02	0.60 ± 0.00[b]	0.60 ± 0.02[b]	0.54 ± 0.02*	0.64 ± 0.13
Day 23	0.66 ± 0.02	0.67 ± 0.02	0.69 ± 0.02	0.66 ± 0.02	0.63 ± 0.02	
Week 14	0.71 ± 0.02	0.74 ± 0.02	0.72 ± 0.01	0.71 ± 0.02	0.67 ± 0.02	
Total protein (g/dL)						
Day 4	5.6 ± 0.2	5.9 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	4.4 ± 0.2**
Day 23	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	5.9 ± 0.0	6.0 ± 0.1	
Week 14	6.7 ± 0.1	6.6 ± 0.1	6.4 ± 0.1*	6.4 ± 0.1*	6.3 ± 0.1**	
Albumin (g/dL)						
Day 4	4.5 ± 0.0	4.6 ± 0.1	4.3 ± 0.1*	4.3 ± 0.1	3.8 ± 0.1**	2.8 ± 0.2**
Day 23	4.6 ± 0.0	4.6 ± 0.1	4.5 ± 0.0	4.4 ± 0.0*	4.2 ± 0.0**	
Week 14	5.1 ± 0.1	5.0 ± 0.1	4.8 ± 0.1*	4.8 ± 0.1**	4.4 ± 0.0**	
Alanine aminotransferase (IU/L)						
Day 4	60 ± 2	51 ± 2	58 ± 2	54 ± 1	189 ± 34*	980 ± 2,251*
Day 23	53 ± 1	51 ± 1	53 ± 2	53 ± 2	61 ± 2	
Week 14	71 ± 3	75 ± 6	75 ± 5	69 ± 4	95 ± 10	
Alkaline phosphatase (IU/L)						
Day 4	1,157 ± 26[b]	1,150 ± 53	1,090 ± 24	1,104 ± 28	1,073 ± 32	1,473 ± 200
Day 23	842 ± 12	819 ± 16	819 ± 18	783 ± 13*	824 ± 17	
Week 14	439 ± 15	473 ± 17	458 ± 11	460 ± 14	505 ± 17*	
Creatine kinase (IU/L)						
Day 4	484 ± 121	216 ± 31*	332 ± 82	337 ± 42	253 ± 23	452 ± 102
Day 23	534 ± 56	496 ± 64	507 ± 28	410 ± 29	646 ± 162	
Week 14	176 ± 15	209 ± 35[b]	215 ± 32	176 ± 22	197 ± 17	
Sorbitol dehydrogenase (IU/L)						
Day 4	17 ± 1	12 ± 1	14 ± 1	15 ± 1	72 ± 19*	588 ± 366*
Day 23	20 ± 1	21 ± 1	23 ± 1	20 ± 1	24 ± 3	
Week 14	18 ± 1	22 ± 2	21 ± 2	20 ± 2	42 ± 7**	
Bile acids (μmol/L)						
Day 4	24.9 ± 3.9	18.8 ± 1.8	19.6 ± 1.8	20.4 ± 1.2	100.2 ± 15.6**	256.3 ± 82.5**
Day 23	16.8 ± 1.9	17.6 ± 3.0	20.4 ± 1.9	21.0 ± 2.4	43.9 ± 4.7**	
Week 14	23.3 ± 2.2	26.6 ± 2.7	25.5 ± 4.2	27.1 ± 1.8	81.0 ± 12.6**	

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data were available for the 100 mg/kg males or females on day 23 or at week 14 due to 100% mortality.
[b] n=9

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Male						
Hematology						
n						
Day 4	10	10	9	10	10	10
Day 23	8	10	10	9	9	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 4	41.3 ± 0.4	42.6 ± 0.8	41.8 ± 0.4	44.1 ± 0.8	40.6 ± 0.4	40.8 ± 0.5
Day 23	43.9 ± 0.4	43.8 ± 0.5	43.2 ± 0.4	44.1 ± 0.3	42.9 ± 0.4	43.8 ± 0.7
Week 14	47.0 ± 0.3	45.7 ± 0.6	47.3 ± 0.7	46.7 ± 0.5	46.5 ± 0.6	47.2 ± 0.4
Hemoglobin (g/dL)						
Day 4	13.5 ± 0.1	13.8 ± 0.2	13.7 ± 0.2	14.3 ± 0.3	13.1 ± 0.2	13.3 ± 0.1
Day 23	14.6 ± 0.1	14.8 ± 0.1	14.6 ± 0.1	14.7 ± 0.1	14.4 ± 0.2	14.6 ± 0.2
Week 14	15.5 ± 0.1	15.0 ± 0.2	15.5 ± 0.2	15.2 ± 0.1	15.3 ± 0.2	15.5 ± 0.1
Erythrocytes (10^6/μL)						
Day 4	6.87 ± 0.07	7.09 ± 0.10	7.02 ± 0.08	7.35 ± 0.15*	6.83 ± 0.07	6.85 ± 0.06
Day 23	7.21 ± 0.09	7.22 ± 0.09	7.17 ± 0.09	7.39 ± 0.06	7.12 ± 0.08	7.24 ± 0.10
Week 14	8.71 ± 0.06	8.50 ± 0.10	8.85 ± 0.13	8.66 ± 0.08	8.72 ± 0.11	8.94 ± 0.08
Reticulocytes (10^6/μL)						
Day 4	0.38 ± 0.02	0.38 ± 0.01	0.38 ± 0.02	0.37 ± 0.02	0.38 ± 0.02	0.36 ± 0.02
Day 23	0.20 ± 0.02	0.21 ± 0.01	0.16 ± 0.01	0.19 ± 0.01	0.20 ± 0.02	0.20 ± 0.01
Week 14	0.11 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01
Nucleated erythrocytes (10^3/μL)						
Day 4	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)						
Day 4	60.3 ± 0.2	60.2 ± 0.3	59.8 ± 0.2	60.2 ± 0.2	59.3 ± 0.3*	59.4 ± 0.3*
Day 23	61.0 ± 0.3	60.9 ± 0.2	60.5 ± 0.4	59.8 ± 0.5	60.3 ± 0.2	60.6 ± 0.4
Week 14	53.9 ± 0.2	54.0 ± 0.2	53.4 ± 0.3	54.1 ± 0.2	53.3 ± 0.2	53.0 ± 0.1**
Mean cell hemoglobin (pg)						
Day 4	19.7 ± 0.2	19.5 ± 0.1	19.5 ± 0.1	19.5 ± 0.2	19.1 ± 0.2	19.3 ± 0.1
Day 23	20.3 ± 0.1	20.5 ± 0.1	20.3 ± 0.2	19.9 ± 0.1	20.2 ± 0.1	20.2 ± 0.1
Week 14	17.8 ± 0.1	17.7 ± 0.1	17.6 ± 0.1	17.5 ± 0.1	17.6 ± 0.1	17.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.8 ± 0.2	32.5 ± 0.2	32.8 ± 0.2	32.4 ± 0.2	32.2 ± 0.4	32.5 ± 0.2
Day 23	33.3 ± 0.2	33.7 ± 0.2	33.6 ± 0.2	33.3 ± 0.1	33.5 ± 0.2	33.3 ± 0.1
Week 14	33.0 ± 0.2	32.9 ± 0.2	32.8 ± 0.2	32.5 ± 0.2	33.0 ± 0.1	32.8 ± 0.2
Platelets (10^3/μL)						
Day 4	839.5 ± 15.2	861.5 ± 25.0	868.0 ± 13.9	906.4 ± 21.8	843.9 ± 17.2	845.7 ± 31.3
Day 23	735.3 ± 7.0	737.5 ± 9.1	753.5 ± 6.6	797.4 ± 12.2**	779.0 ± 9.6**	769.1 ± 16.1**
Week 14	634.9 ± 13.5	654.0 ± 17.1	628.0 ± 22.3	653.2 ± 14.9	668.6 ± 17.0	701.5 ± 34.0*
Leukocytes (10^3/μL)						
Day 4	8.32 ± 0.31	8.41 ± 0.59	8.72 ± 0.38	9.04 ± 0.38	6.96 ± 0.51	8.95 ± 0.39
Day 23	10.64 ± 0.62	9.77 ± 0.48	9.90 ± 0.49	9.21 ± 0.44	7.41 ± 0.59**	9.34 ± 0.41*
Week 14	9.44 ± 0.32	8.79 ± 0.53	9.82 ± 0.70	9.45 ± 0.51	9.01 ± 0.42	8.47 ± 0.50
Segmented neutrophils (10^3/μL)						
Day 4	1.18 ± 0.06	1.23 ± 0.16	1.41 ± 0.10	1.52 ± 0.09	0.91 ± 0.10	1.49 ± 0.08
Day 23	1.24 ± 0.19	1.33 ± 0.13	1.25 ± 0.13	1.37 ± 0.20	1.07 ± 0.15	1.18 ± 0.11
Week 14	2.14 ± 0.13	1.82 ± 0.11	1.97 ± 0.19	1.68 ± 0.11*	1.49 ± 0.14**	1.56 ± 0.11**

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Allyl Alcohol

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	10	10	9	10	10	10
Day 23	8	10	10	9	9	10
Week 14	10	10	10	10	10	10
Lymphocytes (10^3/μL)						
Day 4	6.99 ± 0.35	7.06 ± 0.52	7.14 ± 0.31	7.40 ± 0.38	5.92 ± 0.44	7.21 ± 0.33
Day 23	9.21 ± 0.47	8.25 ± 0.43	8.45 ± 0.42	7.72 ± 0.34*	6.18 ± 0.51**	8.02 ± 0.43*
Week 14	6.98 ± 0.32	6.72 ± 0.48	7.52 ± 0.62	7.56 ± 0.47	7.18 ± 0.42	6.69 ± 0.46
Monocytes (10^3/μL)						
Day 4	0.11 ± 0.03	0.09 ± 0.03	0.13 ± 0.02	0.09 ± 0.03	0.09 ± 0.04	0.21 ± 0.03
Day 23	0.17 ± 0.04	0.17 ± 0.04	0.12 ± 0.03	0.07 ± 0.02	0.13 ± 0.05	0.09 ± 0.02
Week 14	0.22 ± 0.04	0.19 ± 0.03	0.21 ± 0.06	0.14 ± 0.04	0.24 ± 0.06	0.18 ± 0.06
Eosinophils (10^3/μL)						
Day 4	0.04 ± 0.02	0.04 ± 0.03	0.05 ± 0.03	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.02
Day 23	0.02 ± 0.02	0.03 ± 0.02	0.07 ± 0.03	0.05 ± 0.02	0.02 ± 0.02	0.05 ± 0.02
Week 14	0.11 ± 0.03	0.07 ± 0.01	0.11 ± 0.03	0.08 ± 0.02	0.10 ± 0.02	0.04 ± 0.02
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	11.1 ± 0.3	11.4 ± 0.3	11.6 ± 0.5	13.7 ± 0.8	10.3 ± 0.4	9.7 ± 0.4
Day 23	10.2 ± 0.2	11.1 ± 0.4	11.8 ± 0.4**	11.7 ± 0.2**	10.6 ± 0.4	10.7 ± 0.2
Week 14	14.6 ± 0.4	16.2 ± 0.5	14.9 ± 0.4	14.9 ± 0.5	15.4 ± 0.4	16.4 ± 0.2*
Creatinine (mg/dL)						
Day 4	0.60 ± 0.00	0.59 ± 0.01	0.58 ± 0.01	0.59 ± 0.01	0.57 ± 0.02	0.58 ± 0.01
Day 23	0.68 ± 0.01	0.66 ± 0.02	0.69 ± 0.01	0.65 ± 0.02	0.69 ± 0.01	0.65 ± 0.02
Week 14	0.74 ± 0.02	0.72 ± 0.02	0.74 ± 0.02	0.70 ± 0.00	0.78 ± 0.03	0.67 ± 0.02
Total protein (g/dL)						
Day 4	5.6 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	6.0 ± 0.1	5.6 ± 0.1	5.5 ± 0.1
Day 23	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.5 ± 0.1
Week 14	6.8 ± 0.1	6.5 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.1
Albumin (g/dL)						
Day 4	4.3 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.3 ± 0.0	4.1 ± 0.1
Day 23	4.9 ± 0.1	4.7 ± 0.1	4.8 ± 0.0	4.9 ± 0.1	4.8 ± 0.1	4.7 ± 0.0
Week 14	4.8 ± 0.1	4.7 ± 0.0	4.9 ± 0.1	4.8 ± 0.0	4.8 ± 0.1	4.7 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	68 ± 2	65 ± 1	68 ± 2	56 ± 3*	69 ± 3	68 ± 2
Day 23	66 ± 2	60 ± 1	60 ± 2	62 ± 2	59 ± 1*	62 ± 1
Week 14	106 ± 7	112 ± 10	92 ± 5	108 ± 12	104 ± 10	102 ± 11
Alkaline phosphatase (IU/L)						
Day 4	1,647 ± 40	1,654 ± 46	1,686 ± 37	1,598 ± 34	1,607 ± 56	1,574 ± 37
Day 23	1,179 ± 9	1,204 ± 32	1,173 ± 22	1,217 ± 16	1,183 ± 30	1,105 ± 15*
Week 14	611 ± 14	616 ± 11	623 ± 20	599 ± 15	548 ± 10**	474 ± 13**
Creatine kinase (IU/L)						
Day 4	436 ± 42	380 ± 49	479 ± 53	420 ± 45	478 ± 66	365 ± 22
Day 23	305 ± 56	286 ± 40	306 ± 32	407 ± 84	315 ± 37	295 ± 38
Week 14	246 ± 43[b]	383 ± 74	281 ± 65	310 ± 77	193 ± 33	165 ± 29[c]

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Allyl Alcohol

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Sorbitol dehydrogenase (IU/L)						
Day 4	17 ± 0	16 ± 1	18 ± 1	17 ± 1	18 ± 1	16 ± 1
Day 23	18 ± 1	17 ± 1	18 ± 1	18 ± 1	18 ± 1	17 ± 1
Week 14	41 ± 5	44 ± 5	33 ± 3	43 ± 6	43 ± 5	40 ± 5
Bile acids (µmol/L)						
Day 4	20.3 ± 1.0	22.6 ± 2.3	21.2 ± 1.3	22.5 ± 1.7	26.0 ± 2.3*	27.5 ± 1.8**
Day 23	19.2 ± 1.3	21.5 ± 2.1	19.5 ± 2.1	15.9 ± 0.9	25.2 ± 3.0	44.1 ± 4.4**
Week 14	20.5 ± 0.8	21.8 ± 1.0	22.2 ± 1.6	25.1 ± 2.1	29.3 ± 2.3**	29.0 ± 4.0**
Female						
Hematology						
n						
Day 4	10	9	10	10	10	10
Day 23	10	10	9	10	10	8
Week 14	10	10	10	9	10	10
Hematocrit (%)						
Day 4	43.3 ± 0.4	43.3 ± 0.4	43.6 ± 0.9	42.8 ± 0.6	43.2 ± 0.5	42.6 ± 0.5
Day 23	43.5 ± 0.4	43.9 ± 0.4	43.8 ± 0.4	44.1 ± 0.5	43.9 ± 0.6	45.3 ± 0.2*
Week 14	45.2 ± 0.4	45.2 ± 0.7	45.5 ± 0.6	46.3 ± 0.6	45.8 ± 0.5	45.6 ± 0.4
Hemoglobin (g/dL)						
Day 4	14.3 ± 0.1	14.5 ± 0.1	14.4 ± 0.3	14.3 ± 0.2	14.3 ± 0.3	14.1 ± 0.2
Day 23	14.6 ± 0.1	14.6 ± 0.1	14.7 ± 0.1	14.7 ± 0.1	14.5 ± 0.2	14.9 ± 0.1
Week 14	14.8 ± 0.1	14.9 ± 0.2	14.7 ± 0.2	14.9 ± 0.1	14.8 ± 0.1	14.8 ± 0.1
Erythrocytes (10^6/µL)						
Day 4	7.21 ± 0.08	7.21 ± 0.06	7.19 ± 0.15	7.09 ± 0.11	7.14 ± 0.07	7.10 ± 0.08
Day 23	7.03 ± 0.06	7.10 ± 0.07	7.06 ± 0.07	7.14 ± 0.09	7.10 ± 0.12	7.29 ± 0.05
Week 14	7.83 ± 0.07	7.89 ± 0.12	7.86 ± 0.11	8.03 ± 0.08	7.89 ± 0.07	7.99 ± 0.06
Reticulocytes (10^6/µL)						
Day 4	0.22 ± 0.02	0.22 ± 0.01	0.25 ± 0.02	0.22 ± 0.02	0.23 ± 0.02	0.20 ± 0.01
Day 23	0.10 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01
Week 14	0.12 ± 0.01	0.14 ± 0.02	0.14 ± 0.02	0.12 ± 0.01	0.12 ± 0.01	0.16 ± 0.01
Nucleated erythrocytes (10^3/µL)						
Day 4	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.04 ± 0.03
Day 23	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[c]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)						
Day 4	60.3 ± 0.3	60.1 ± 0.3	60.8 ± 0.2	60.3 ± 0.3	60.7 ± 0.3	60.0 ± 0.3
Day 23	61.9 ± 0.3	61.9 ± 0.2	62.2 ± 0.4	61.7 ± 0.4	62.0 ± 0.3	62.3 ± 0.3
Week 14	57.8 ± 0.3	57.4 ± 0.2	57.8 ± 0.2	57.7 ± 0.3	58.2 ± 0.2	57.1 ± 0.3
Mean cell hemoglobin (pg)						
Day 4	19.9 ± 0.1	20.1 ± 0.1	20.0 ± 0.1	20.2 ± 0.1	20.1 ± 0.2	19.9 ± 0.1
Day 23	20.7 ± 0.1	20.6 ± 0.1	20.8 ± 0.2	20.6 ± 0.1	20.4 ± 0.2	20.4 ± 0.1
Week 14	18.9 ± 0.1	18.9 ± 0.1	18.7 ± 0.1	18.5 ± 0.2	18.8 ± 0.1	18.5 ± 0.1

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Allyl Alcohol

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	9	10	10	10	10
Day 23	10	10	9	10	10	8
Week 14	10	10	10	9	10	10
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.0 ± 0.2	33.5 ± 0.2	33.0 ± 0.1	33.5 ± 0.2	33.1 ± 0.4	33.2 ± 0.1
Day 23	33.5 ± 0.2	33.3 ± 0.2	33.5 ± 0.3	33.3 ± 0.3	32.9 ± 0.2	32.8 ± 0.2
Week 14	32.8 ± 0.2	33.0 ± 0.3	32.5 ± 0.3	32.2 ± 0.4	32.3 ± 0.3	32.5 ± 0.2
Platelets (10^3/μL)						
Day 4	859.6 ± 54.0	880.1 ± 51.1	914.8 ± 21.1	941.7 ± 25.6	905.2 ± 29.4	915.1 ± 22.3
Day 23	731.3 ± 9.1	747.7 ± 12.2	752.2 ± 11.6	731.5 ± 14.3	750.3 ± 13.1	716.1 ± 26.9
Week 14	688.0 ± 14.0	661.6 ± 32.8	680.4 ± 14.8	730.7 ± 28.1	669.9 ± 19.6	742.6 ± 14.2
Leukocytes (10^3/μL)						
Day 4	7.98 ± 0.48	8.70 ± 0.57	8.51 ± 0.46	7.92 ± 0.55	8.88 ± 0.60	8.59 ± 0.69
Day 23	9.35 ± 0.62	9.26 ± 0.38	9.34 ± 0.61	8.21 ± 0.35	9.07 ± 0.41	8.71 ± 0.50
Week 14	6.52 ± 0.45	6.80 ± 0.44	6.18 ± 0.58	6.86 ± 0.48	6.68 ± 0.39	6.49 ± 0.44
Segmented neutrophils (10^3/μL)						
Day 4	1.03 ± 0.11	0.93 ± 0.12	1.38 ± 0.12	1.01 ± 0.12	1.24 ± 0.13	1.20 ± 0.12
Day 23	1.20 ± 0.10	0.97 ± 0.07	1.03 ± 0.12	0.77 ± 0.10*	1.06 ± 0.12	1.08 ± 0.15
Week 14	1.15 ± 0.08	1.37 ± 0.14	1.10 ± 0.12	1.03 ± 0.08	1.28 ± 0.12	1.30 ± 0.19
Lymphocytes (10^3/μL)						
Day 4	6.83 ± 0.43	7.67 ± 0.51	7.00 ± 0.38	6.77 ± 0.45	7.52 ± 0.51	7.29 ± 0.63
Day 23	7.90 ± 0.54	8.10 ± 0.36	8.04 ± 0.63	7.33 ± 0.31	7.81 ± 0.44	7.47 ± 0.50
Week 14	5.20 ± 0.44	5.25 ± 0.39	4.88 ± 0.47	5.68 ± 0.47	5.24 ± 0.38	5.05 ± 0.35
Monocytes (10^3/μL)						
Day 4	0.10 ± 0.04	0.05 ± 0.01	0.10 ± 0.03	0.07 ± 0.02	0.08 ± 0.03	0.07 ± 0.03
Day 23	0.13 ± 0.04	0.11 ± 0.03	0.17 ± 0.04	0.07 ± 0.02	0.11 ± 0.04	0.11 ± 0.05
Week 14	0.13 ± 0.03	0.13 ± 0.02	0.13 ± 0.03	0.10 ± 0.03	0.13 ± 0.03	0.12 ± 0.02
Eosinophils (10^3/μL)						
Day 4	0.02 ± 0.01	0.05 ± 0.02	0.04 ± 0.02	0.08 ± 0.02	0.04 ± 0.02	0.03 ± 0.02
Day 23	0.12 ± 0.05	0.08 ± 0.03	0.11 ± 0.03	0.04 ± 0.01	0.08 ± 0.02	0.05 ± 0.02
Week 14	0.05 ± 0.01	0.06 ± 0.02	0.07 ± 0.03	0.05 ± 0.02	0.03 ± 0.02	0.02 ± 0.02
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	9
Week 14	10	10	10	9	10	10
Urea nitrogen (mg/dL)						
Day 4	11.8 ± 0.7	11.7 ± 0.5	12.3 ± 0.8	12.4 ± 0.4	12.5 ± 0.4	10.9 ± 0.5
Day 23	13.3 ± 0.4	13.0 ± 0.4	13.5 ± 0.4	13.6 ± 0.3	12.6 ± 0.3	13.4 ± 0.4
Week 14	15.4 ± 0.5	16.8 ± 0.7	15.0 ± 0.8	15.8 ± 0.5	14.4 ± 0.3	16.7 ± 0.7
Creatinine (mg/dL)						
Day 4	0.58 ± 0.01	0.58 ± 0.01	0.60 ± 0.00	0.61 ± 0.01	0.57 ± 0.02	0.60 ± 0.00
Day 23	0.63 ± 0.02	0.62 ± 0.01	0.65 ± 0.02	0.65 ± 0.02	0.63 ± 0.02	0.67 ± 0.02
Week 14	0.76 ± 0.03	0.76 ± 0.02	0.71 ± 0.02	0.70 ± 0.02	0.69 ± 0.02*	0.68 ± 0.02*

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 13-Week Gavage Study of Allyl Alcohol

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	9
Week 14	10	10	10	9	10	10
Total protein (g/dL)						
Day 4	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1
Day 23	6.1 ± 0.0	6.1 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.2 ± 0.1
Week 14	6.8 ± 0.1	6.8 ± 0.2	6.7 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.6 ± 0.1
Albumin (g/dL)						
Day 4	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.4 ± 0.1
Day 23	4.6 ± 0.0	4.7 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.6 ± 0.0
Week 14	5.1 ± 0.1	5.1 ± 0.1	5.0 ± 0.1	4.9 ± 0.1*	4.9 ± 0.1	4.7 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 4	59 ± 2	57 ± 1	56 ± 2	60 ± 2	56 ± 2	61 ± 2
Day 23	47 ± 2	55 ± 1*	52 ± 1	55 ± 2*	52 ± 1	56 ± 2**
Week 14	82 ± 5	79 ± 4	85 ± 6	78 ± 4	70 ± 3	74 ± 9
Alkaline phosphatase (IU/L)						
Day 4	1,199 ± 21	1,231 ± 35	1,265 ± 29	1,207 ± 27	1,242 ± 38	1,180 ± 18
Day 23	837 ± 19	827 ± 22	833 ± 18	823 ± 15	800 ± 14	818 ± 17
Week 14	464 ± 16	457 ± 13	479 ± 23	442 ± 14	425 ± 33*	413 ± 11*
Creatine kinase (IU/L)						
Day 4	395 ± 71[c]	600 ± 160	336 ± 63	486 ± 54	437 ± 98	302 ± 33
Day 23	287 ± 30	288 ± 47	336 ± 68	321 ± 39	341 ± 43	537 ± 124
Week 14	208 ± 30	284 ± 47	193 ± 30[c]	222 ± 78	154 ± 20	197 ± 66
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 1	17 ± 1	16 ± 1	17 ± 1	16 ± 1	16 ± 1
Day 23	16 ± 1	17 ± 1	17 ± 1	17 ± 1	17 ± 1	20 ± 1*
Week 14	23 ± 2	26 ± 3	30 ± 4	26 ± 2	23 ± 2	24 ± 6
Bile acids (μmol/L)						
Day 4	18.6 ± 0.8	21.3 ± 1.8	16.7 ± 0.9	18.5 ± 1.4	16.2 ± 1.0	18.9 ± 1.1
Day 23	22.3 ± 2.6	25.3 ± 3.4	20.7 ± 4.1	21.1 ± 1.8	26.8 ± 2.9	26.7 ± 2.5
Week 14	25.8 ± 1.9	30.0 ± 4.0	33.5 ± 3.1	29.3 ± 1.6	37.5 ± 3.3*	45.8 ± 15.8

* 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=8
[c] n=9

TABLE C3
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Acrolein[a]

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	9	10	9
Day 23	10	10	10	9	10	7
Week 14	10	10	9	7	8	1[b]
Hematocrit (%)						
Day 4	43.9 ± 0.5	43.8 ± 0.5	43.1 ± 0.8	43.7 ± 0.6	44.9 ± 0.9	48.3 ± 1.2**
Day 23	44.7 ± 0.3	44.8 ± 0.4	45.2 ± 0.5	45.6 ± 0.5	47.8 ± 1.2**	46.1 ± 1.9
Week 14	46.5 ± 0.4	47.5 ± 0.3	46.6 ± 0.5	47.1 ± 0.3	48.4 ± 0.5*	33.0
Hemoglobin (g/dL)						
Day 4	13.8 ± 0.1	13.9 ± 0.2	13.8 ± 0.2	13.9 ± 0.2	14.3 ± 0.3	15.7 ± 0.3**
Day 23	14.7 ± 0.1	14.7 ± 0.1	14.8 ± 0.1	14.8 ± 0.2	15.5 ± 0.3	14.7 ± 0.6
Week 14	15.1 ± 0.2	15.2 ± 0.1	14.9 ± 0.1	15.2 ± 0.1	15.6 ± 0.3	10.5
Erythrocytes (10^6/µL)						
Day 4	7.18 ± 0.09	7.19 ± 0.09	7.07 ± 0.13	7.22 ± 0.11	7.45 ± 0.13	8.24 ± 0.18**
Day 23	7.31 ± 0.05	7.35 ± 0.07	7.34 ± 0.10	7.43 ± 0.13	7.82 ± 0.18*	8.09 ± 0.30*
Week 14	8.61 ± 0.07	8.71 ± 0.08	8.57 ± 0.12	8.64 ± 0.09	8.69 ± 0.10	5.44
Reticulocytes (10^6/µL)						
Day 4	0.31 ± 0.02	0.34 ± 0.02	0.32 ± 0.02	0.30 ± 0.03	0.33 ± 0.03	0.30 ± 0.02
Day 23	0.16 ± 0.01	0.17 ± 0.02	0.14 ± 0.01	0.18 ± 0.02	0.20 ± 0.02*	0.38 ± 0.04**
Week 14	0.16 ± 0.01	0.15 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.20 ± 0.02	0.85
Nucleated erythrocytes (10^3/µL)						
Day 4	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.06 ± 0.06	0.00 ± 0.00
Day 23	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.06**
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
Mean cell volume (fL)						
Day 4	61.2 ± 0.2	61.1 ± 0.2	61.2 ± 0.4	60.7 ± 0.4	60.4 ± 0.3	58.4 ± 0.4**
Day 23	61.3 ± 0.2	61.0 ± 0.3	61.6 ± 0.3	61.3 ± 0.6	61.2 ± 0.4	56.9 ± 0.8**
Week 14	53.8 ± 0.2	54.5 ± 0.2*	54.3 ± 0.4	54.6 ± 0.3	55.8 ± 0.2**	61.0
Mean cell hemoglobin (pg)						
Day 4	19.3 ± 0.1	19.4 ± 0.1	19.5 ± 0.1	19.3 ± 0.1	19.2 ± 0.1	19.1 ± 0.1
Day 23	20.1 ± 0.1	19.9 ± 0.1	20.2 ± 0.1	19.9 ± 0.2	19.9 ± 0.2	18.1 ± 0.3**
Week 14	17.5 ± 0.1	17.5 ± 0.1	17.4 ± 0.2	17.6 ± 0.1	18.0 ± 0.1*	19.3
Mean cell hemoglobin concentration (g/dL)						
Day 4	31.5 ± 0.2	31.8 ± 0.2	32.0 ± 0.2	31.9 ± 0.2	31.9 ± 0.2	32.6 ± 0.2*
Day 23	32.8 ± 0.2	32.8 ± 0.2	32.8 ± 0.1	32.5 ± 0.2	32.6 ± 0.2	31.8 ± 0.3**
Week 14	32.5 ± 0.3	32.0 ± 0.1	32.1 ± 0.2	32.3 ± 0.1	32.2 ± 0.2	31.8
Platelets (10^3/µL)						
Day 4	918.4 ± 17.8	906.1 ± 23.0	914.4 ± 32.9	980.0 ± 24.5*	1,043.1 ± 33.3**	1,172.4 ± 38.4**
Day 23	743.4 ± 7.1	747.3 ± 16.3	737.4 ± 9.2	729.7 ± 21.3	832.1 ± 14.0**	980.7 ± 59.8**
Week 14	626.4 ± 11.6	648.4 ± 11.2	645.8 ± 10.9	651.4 ± 14.6	754.6 ± 14.3**	1,186.0
Leukocytes (10^3/µL)						
Day 4	9.05 ± 0.41	9.40 ± 0.43	10.58 ± 0.53*	9.98 ± 0.54	12.19 ± 0.80**	12.29 ± 0.80**
Day 23	10.12 ± 0.43	8.71 ± 0.47	9.75 ± 0.78	9.81 ± 0.96	10.29 ± 0.46	10.01 ± 0.99
Week 14	11.00 ± 0.65	10.54 ± 0.52	10.12 ± 0.44	10.77 ± 0.55	11.69 ± 0.69	7.20
Segmented neutrophils (10^3/µL)						
Day 4	1.15 ± 0.13	1.14 ± 0.15	1.20 ± 0.15	1.41 ± 0.10	2.18 ± 0.35*	3.13 ± 0.36**
Day 23	1.05 ± 0.16	1.26 ± 0.14	1.21 ± 0.13	1.04 ± 0.18	2.00 ± 0.34**	2.77 ± 0.34**
Week 14	2.25 ± 0.28	1.90 ± 0.17	2.19 ± 0.12	1.95 ± 0.25	2.65 ± 0.28	2.09

TABLE C3
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Acrolein

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	9	10	9
Day 23	10	10	10	9	10	7
Week 14	10	10	9	7	8	1
Lymphocytes (10^3/μL)						
Day 4	7.66 ± 0.40	8.02 ± 0.35	9.08 ± 0.53	8.34 ± 0.54	9.66 ± 0.60	8.61 ± 0.48
Day 23	8.75 ± 0.50	7.20 ± 0.38	8.18 ± 0.72	8.28 ± 0.83	7.91 ± 0.38	6.92 ± 1.11
Week 14	8.38 ± 0.58	8.26 ± 0.43	7.58 ± 0.45	8.32 ± 0.59	8.75 ± 0.48	4.90
Monocytes (10^3/μL)						
Day 4	0.18 ± 0.03	0.19 ± 0.05	0.24 ± 0.04	0.19 ± 0.03	0.31 ± 0.06	0.51 ± 0.09**
Day 23	0.31 ± 0.05	0.22 ± 0.04	0.28 ± 0.06	0.45 ± 0.13	0.33 ± 0.05	0.30 ± 0.09
Week 14	0.31 ± 0.07	0.25 ± 0.03	0.25 ± 0.07	0.33 ± 0.04	0.24 ± 0.08	0.14
Eosinophils (10^3/μL)						
Day 4	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.03	0.04 ± 0.02
Day 23	0.01 ± 0.01	0.03 ± 0.02	0.09 ± 0.03	0.04 ± 0.02	0.05 ± 0.02	0.03 ± 0.03
Week 14	0.07 ± 0.03	0.13 ± 0.04	0.10 ± 0.04	0.17 ± 0.07	0.05 ± 0.03	0.07
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	9
Day 23	10	10	10	9	10	7
Week 14	10	10	10	8	8	1
Urea nitrogen (mg/dL)						
Day 4	9.8 ± 0.4	10.2 ± 0.3	10.6 ± 0.5	10.2 ± 0.4	11.7 ± 0.7*	12.9 ± 1.2**
Day 23	11.8 ± 0.4	12.4 ± 0.4	11.5 ± 0.5	12.6 ± 0.4	15.5 ± 1.1**	15.6 ± 1.6**
Week 14	15.4 ± 0.5	15.4 ± 0.3	15.2 ± 0.4	18.0 ± 0.4**	18.1 ± 0.7**	16.0
Creatinine (mg/dL)						
Day 4	0.53 ± 0.02	0.56 ± 0.02	0.58 ± 0.01	0.53 ± 0.02	0.54 ± 0.02	0.50 ± 0.00
Day 23	0.64 ± 0.02	0.64 ± 0.02	0.61 ± 0.01	0.61 ± 0.01	0.61 ± 0.01	0.59 ± 0.01*
Week 14	0.74 ± 0.02	0.74 ± 0.02	0.74 ± 0.02	0.70 ± 0.00	0.70 ± 0.02	0.70
Total protein (g/dL)						
Day 4	5.8 ± 0.0	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.5 ± 0.1	4.6 ± 0.1**
Day 23	6.0 ± 0.0	6.0 ± 0.0	6.1 ± 0.0	5.8 ± 0.0**	5.4 ± 0.2**	5.0 ± 0.3**
Week 14	6.8 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.5 ± 0.1**	5.9 ± 0.2**	5.1
Albumin (g/dL)						
Day 4	4.3 ± 0.0	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1*	3.9 ± 0.1**	3.2 ± 0.1**
Day 23	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.2 ± 0.0**	3.9 ± 0.2**	3.6 ± 0.2**
Week 14	4.8 ± 0.1	4.8 ± 0.0	4.8 ± 0.0	4.7 ± 0.1	4.2 ± 0.1**	3.6
Alanine aminotransferase (IU/L)						
Day 4	67 ± 2	74 ± 2	73 ± 2	69 ± 2	66 ± 3	52 ± 3*
Day 23	60 ± 1	61 ± 2	60 ± 2	60 ± 2	59 ± 4	60 ± 4
Week 14	94 ± 7	105 ± 5	107 ± 8	104 ± 7	97 ± 10	217
Alkaline phosphatase (IU/L)						
Day 4	1,628 ± 27	1,667 ± 47	1,648 ± 33	1,476 ± 31*	1,187 ± 73**	750 ± 71**
Day 23	1,189 ± 25	1,212 ± 23	1,214 ± 28	1,093 ± 22*	923 ± 32**	760 ± 59**
Week 14	628 ± 17	663 ± 13	593 ± 15	620 ± 14	479 ± 19**	440

TABLE C3
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Acrolein

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	9
Day 23	10	10	10	9	10	7
Week 14	10	10	10	8	8	1
Creatine kinase (IU/L)						
Day 4	248 ± 17	315 ± 37	280 ± 22	298 ± 40	250 ± 21	258 ± 36
Day 23	329 ± 35	360 ± 52	341 ± 40	403 ± 44	373 ± 33	357 ± 67[c]
Week 14	159 ± 19	178 ± 21	178 ± 33	138 ± 12	162 ± 21	5,320
Sorbitol dehydrogenase (IU/L)						
Day 4	17 ± 1	15 ± 1	16 ± 1	15 ± 1	18 ± 1	17 ± 1
Day 23	22 ± 1	22 ± 1	24 ± 1	22 ± 2	22 ± 1	19 ± 2
Week 14	32 ± 4	36 ± 3	42 ± 5	28 ± 2	40 ± 5	14
Bile salts (μmol/L)						
Day 4	22.0 ± 1.2	24.6 ± 2.5	24.7 ± 1.5	21.5 ± 1.7	26.4 ± 1.6	29.8 ± 2.4*
Day 23	21.0 ± 2.1	16.9 ± 0.9	23.0 ± 3.3	20.0 ± 1.6	17.4 ± 2.1	25.7 ± 4.1
Week 14	19.1 ± 1.6	17.9 ± 1.2	23.6 ± 2.2	19.9 ± 1.7	19.1 ± 1.4	28.0
Female						
Hematology						
n						
Day 4	10	9	9	10	10	10
Day 23	10	10	10	10	10	6
Week 14	9	10	7	7	9	2
Hematocrit (%)						
Day 4	47.2 ± 1.0	46.0 ± 1.3	45.3 ± 0.9	45.7 ± 0.9	46.9 ± 0.7	52.2 ± 1.4
Day 23	46.5 ± 0.5	47.3 ± 0.6	46.7 ± 0.4	47.8 ± 0.5	50.1 ± 0.7**	49.1 ± 1.1*
Week 14	46.8 ± 0.3	46.1 ± 0.5	47.8 ± 0.3	47.2 ± 0.4	48.4 ± 0.8	49.6 ± 7.8
Hemoglobin (g/dL)						
Day 4	14.8 ± 0.3	14.7 ± 0.4	14.2 ± 0.2	14.6 ± 0.2	14.9 ± 0.3	16.7 ± 0.5**
Day 23	14.8 ± 0.1	15.1 ± 0.2	14.9 ± 0.1	15.2 ± 0.2	15.9 ± 0.2**	15.3 ± 0.4*
Week 14	15.0 ± 0.1	14.9 ± 0.1	15.2 ± 0.1	15.0 ± 0.1	15.6 ± 0.3	15.9 ± 2.6
Erythrocytes (10^6/μL)						
Day 4	7.55 ± 0.17	7.45 ± 0.21	7.35 ± 0.14	7.39 ± 0.12	7.65 ± 0.14	8.68 ± 0.23**
Day 23	7.43 ± 0.09	7.63 ± 0.10	7.53 ± 0.06	7.68 ± 0.09	8.09 ± 0.12**	8.12 ± 0.25**
Week 14	8.04 ± 0.06	7.96 ± 0.07	8.23 ± 0.04	8.07 ± 0.05	8.31 ± 0.11	8.19 ± 1.52
Reticulocytes (10^6/μL)						
Day 4	0.24 ± 0.02	0.24 ± 0.02	0.26 ± 0.01	0.26 ± 0.02	0.24 ± 0.02	0.29 ± 0.03
Day 23	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.32 ± 0.04**
Week 14	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.16 ± 0.02**	0.38 ± 0.07**
Nucleated erythrocytes (10^3/μL)						
Day 4	0.00 ± 0.00	0.01 ± 0.01	0.06 ± 0.03	0.07 ± 0.04	0.03 ± 0.02	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00

TABLE C3
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Acrolein

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	9	9	10	10	10
Day 23	10	10	10	10	10	6
Week 14	9	10	7	7	9	2
Mean cell volume (fL)						
Day 4	62.4 ± 0.3	61.9 ± 0.3	61.6 ± 0.3*	61.8 ± 0.5	61.5 ± 0.3	60.1 ± 0.4**
Day 23	62.5 ± 0.3	61.9 ± 0.2	62.2 ± 0.3	62.3 ± 0.3	61.8 ± 0.2	60.7 ± 0.8*
Week 14	58.3 ± 0.2	57.9 ± 0.2	58.3 ± 0.2	58.6 ± 0.3	58.3 ± 0.2	61.0 ± 2.0
Mean cell hemoglobin (pg)						
Day 4	19.6 ± 0.1	19.8 ± 0.1	19.4 ± 0.1	19.8 ± 0.1	19.5 ± 0.1	19.3 ± 0.1
Day 23	20.0 ± 0.2	19.9 ± 0.1	19.9 ± 0.2	19.9 ± 0.1	19.6 ± 0.1	18.9 ± 0.2**
Week 14	18.7 ± 0.1	18.7 ± 0.1	18.5 ± 0.1	18.6 ± 0.1	18.8 ± 0.1	19.4 ± 0.5
Mean cell hemoglobin concentration (g/dL)						
Day 4	31.3 ± 0.2	32.0 ± 0.2	31.4 ± 0.2	32.0 ± 0.4	31.7 ± 0.2	32.1 ± 0.2
Day 23	32.0 ± 0.3	32.0 ± 0.1	32.0 ± 0.2	31.9 ± 0.1	31.7 ± 0.2	31.2 ± 0.1
Week 14	32.0 ± 0.2	32.3 ± 0.3	31.8 ± 0.2	31.8 ± 0.3	32.3 ± 0.2	32.0 ± 0.2
Platelets (10^3/μL)						
Day 4	942.9 ± 18.8	940.4 ± 18.5	987.7 ± 32.1	989.5 ± 32.0	1,083.8 ± 35.5**	1,113.4 ± 32.5**
Day 23	760.8 ± 14.9	767.1 ± 17.7	750.5 ± 9.0	795.1 ± 9.0*	890.7 ± 20.9**	1,084.2 ± 45.2**
Week 14	652.4 ± 9.8	645.1 ± 14.5	646.4 ± 16.7	675.3 ± 20.1	770.8 ± 9.5**	1,098.5 ± 117.5*
Leukocytes (10^3/μL)						
Day 4	11.44 ± 0.73	11.71 ± 0.65	10.00 ± 0.28	10.45 ± 0.65	11.01 ± 0.48	12.57 ± 0.95
Day 23	9.73 ± 0.30	10.18 ± 0.62	9.48 ± 0.30	10.56 ± 0.57	12.14 ± 0.66**	11.10 ± 0.54*
Week 14	8.73 ± 0.54	9.15 ± 0.32	8.89 ± 0.45	9.77 ± 0.61	9.81 ± 0.50	9.60 ± 0.80
Segmented neutrophils (10^3/μL)						
Day 4	1.30 ± 0.13	1.45 ± 0.20	1.12 ± 0.14	1.54 ± 0.15	1.75 ± 0.14	3.72 ± 0.70**
Day 23	1.05 ± 0.13	1.23 ± 0.14	0.83 ± 0.08	1.24 ± 0.12	1.64 ± 0.21*	1.96 ± 0.25**
Week 14	1.48 ± 0.15	1.64 ± 0.15	1.58 ± 0.15	1.67 ± 0.24	1.93 ± 0.11*	1.98 ± 0.93
Lymphocytes (10^3/μL)						
Day 4	9.88 ± 0.62	9.95 ± 0.61	8.69 ± 0.28	8.70 ± 0.57	8.99 ± 0.45	8.41 ± 0.56
Day 23	8.33 ± 0.24	8.65 ± 0.54	8.32 ± 0.31	8.92 ± 0.49	10.09 ± 0.62	8.65 ± 0.53
Week 14	7.01 ± 0.44	7.33 ± 0.29	7.07 ± 0.28	7.88 ± 0.51	7.69 ± 0.44	7.52 ± 0.14
Monocytes (10^3/μL)						
Day 4	0.22 ± 0.04	0.20 ± 0.03	0.14 ± 0.04	0.19 ± 0.03	0.25 ± 0.05	0.42 ± 0.10
Day 23	0.29 ± 0.04	0.23 ± 0.03	0.26 ± 0.05	0.26 ± 0.04	0.31 ± 0.04	0.37 ± 0.07
Week 14	0.14 ± 0.03	0.10 ± 0.02	0.14 ± 0.02	0.18 ± 0.03	0.10 ± 0.02	0.10 ± 0.01
Eosinophils (10^3/μL)						
Day 4	0.04 ± 0.02	0.11 ± 0.04	0.05 ± 0.03	0.02 ± 0.02	0.02 ± 0.01	0.01 ± 0.01
Day 23	0.07 ± 0.03	0.07 ± 0.04	0.08 ± 0.03	0.14 ± 0.04	0.11 ± 0.04	0.12 ± 0.05
Week 14	0.11 ± 0.04	0.08 ± 0.02	0.09 ± 0.04	0.05 ± 0.02	0.09 ± 0.03	0.00 ± 0.00

TABLE C3
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Acrolein

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Female (continued)						
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	10	9	10	10	10	6
Week 14	10	10	9	8	9	2
Urea nitrogen (mg/dL)						
Day 4	12.8 ± 0.7	13.1 ± 0.4	11.8 ± 0.6	12.9 ± 0.5	14.3 ± 0.5	14.2 ± 1.7
Day 23	14.1 ± 0.3	14.3 ± 0.4	14.4 ± 0.6	14.0 ± 0.4	15.6 ± 1.0	15.3 ± 1.2
Week 14	14.8 ± 0.5	16.6 ± 0.3**	16.4 ± 0.6*	18.6 ± 0.6**	19.9 ± 1.0**	22.5 ± 2.5**
Creatinine (mg/dL)						
Day 4	0.59 ± 0.01	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00	0.55 ± 0.02*
Day 23	0.67 ± 0.02	0.71 ± 0.01	0.68 ± 0.01	0.65 ± 0.02	0.63 ± 0.02	0.65 ± 0.02
Week 14	0.71 ± 0.01	0.72 ± 0.01	0.70 ± 0.00	0.69 ± 0.01	0.70 ± 0.00	0.65 ± 0.05
Total protein (g/dL)						
Day 4	6.0 ± 0.1	5.9 ± 0.1	5.9 ± 0.0	5.8 ± 0.1	6.1 ± 0.1	4.5 ± 0.2**
Day 23	6.1 ± 0.0	6.3 ± 0.2	6.1 ± 0.0	5.9 ± 0.1	5.7 ± 0.1**	5.5 ± 0.2**
Week 14	6.7 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.4 ± 0.2*	6.1 ± 0.1**	5.0 ± 0.4**
Albumin (g/dL)						
Day 4	4.6 ± 0.1	4.6 ± 0.1	4.5 ± 0.0	4.4 ± 0.0*	4.5 ± 0.1	3.1 ± 0.1**
Day 23	4.5 ± 0.0	4.7 ± 0.1	4.6 ± 0.0	4.4 ± 0.1	4.1 ± 0.1**	3.9 ± 0.1**
Week 14	5.0 ± 0.1[d]	4.8 ± 0.0	5.0 ± 0.1	4.8 ± 0.1	4.6 ± 0.0**	3.6 ± 0.3**
Alanine aminotransferase (IU/L)						
Day 4	60 ± 3	58 ± 2	60 ± 1	60 ± 3	60 ± 3	55 ± 7
Day 23	49 ± 2	50 ± 2	48 ± 1	50 ± 1	49 ± 3	55 ± 4
Week 14	72 ± 5	79 ± 6	63 ± 3	81 ± 11	65 ± 5	50 ± 4
Alkaline phosphatase (IU/L)						
Day 4	1,280 ± 37	1,202 ± 23	1,225 ± 22	1,145 ± 27**	1,005 ± 41**	559 ± 61**
Day 23	880 ± 14	937 ± 66	832 ± 13	804 ± 20**	663 ± 39**	656 ± 18**
Week 14	470 ± 14	495 ± 19	470 ± 18	510 ± 24	373 ± 19**	318 ± 51*
Creatine kinase (IU/L)						
Day 4	310 ± 50	238 ± 22	270 ± 35	333 ± 65	306 ± 76	414 ± 107
Day 23	272 ± 34	297 ± 25	235 ± 41	268 ± 39	279 ± 25	272 ± 46
Week 14	217 ± 63	240 ± 29	194 ± 31	279 ± 70	182 ± 27	167 ± 7
Sorbitol dehydrogenase (IU/L)						
Day 4	17 ± 1	15 ± 1	16 ± 1	17 ± 2	19 ± 3	21 ± 2
Day 23	18 ± 1	24 ± 1**	20 ± 1	19 ± 1	20 ± 1	18 ± 2
Week 14	25 ± 2	29 ± 3	22 ± 1	29 ± 5	23 ± 2	16 ± 1
Bile salts (μm/l)						
Day 4	24.1 ± 3.6	17.3 ± 0.8	22.7 ± 1.7	21.8 ± 1.3	27.0 ± 2.1	25.8 ± 1.7[d]
Day 23	20.3 ± 1.6	17.0 ± 2.1	18.6 ± 1.4	22.0 ± 3.0	19.2 ± 1.9	29.3 ± 6.4
Week 14	16.9 ± 1.7	21.9 ± 3.5	18.8 ± 2.9	27.3 ± 3.0*	19.9 ± 3.7	14.0 ± 0.0

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] No standard error was calculated because fewer than two measurements were available.
[c] n=6
[d] n=9

TABLE C4
Hematology Data for Mice in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	8 mg/kg	16 mg/kg	32 mg/kg	62.5 mg/kg
Male					
n	9	7	10	9	1[b]
Hematocrit (%)	48.9 ± 0.7	51.2 ± 1.7	49.8 ± 0.6	49.8 ± 0.5	52.3
Hemoglobin (g/dL)	16.1 ± 0.2	16.7 ± 0.5	16.3 ± 0.2	16.4 ± 0.2	16.8
Erythrocytes (10^6/μL)	10.23 ± 0.14	10.86 ± 0.34	10.53 ± 0.15	10.50 ± 0.13	11.05
Reticulocytes (10^6/μL)	0.09 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.07
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00
Mean cell volume (fL)	47.8 ± 0.1	47.1 ± 0.1*	47.3 ± 0.2	47.3 ± 0.2	47.0
Mean cell hemoglobin (pg)	15.8 ± 0.1	15.4 ± 0.1*	15.5 ± 0.1	15.6 ± 0.1	15.2
Mean cell hemoglobin concentration (g/dL)	33.0 ± 0.2	32.8 ± 0.2	32.7 ± 0.3	32.9 ± 0.2	32.1
Platelets (10^3/μL)	757.8 ± 33.4	643.0 ± 53.5	678.5 ± 39.0	725.6 ± 46.4	722.0
Leukocytes (10^3/μL)	5.13 ± 0.37	4.11 ± 0.20	4.08 ± 0.21*	4.22 ± 0.22	3.30
Segmented neutrophils (10^3/μL)	0.70 ± 0.07	0.43 ± 0.11	0.52 ± 0.10	0.53 ± 0.06	0.53
Lymphocytes (10^3/μL)	4.27 ± 0.28	3.46 ± 0.20	3.36 ± 0.12*	3.54 ± 0.20	2.54
Monocytes (10^3/μL)	0.02 ± 0.01	0.08 ± 0.01*	0.07 ± 0.02*	0.05 ± 0.02	0.03
Eosinophils (10^3/μL)	0.15 ± 0.04	0.15 ± 0.03	0.12 ± 0.02	0.11 ± 0.02	0.20
Female					
n	9	8	6	7	6
Hematocrit (%)	46.4 ± 0.6	46.8 ± 0.9	46.9 ± 0.3	47.2 ± 0.8	49.3 ± 0.9
Hemoglobin (g/dL)	15.4 ± 0.2	15.4 ± 0.3	15.5 ± 0.1	15.6 ± 0.2	16.0 ± 0.2
Erythrocytes (10^6/μL)	9.81 ± 0.12	9.90 ± 0.18	10.01 ± 0.09	10.03 ± 0.18	10.27 ± 0.17
Reticulocytes (10^6/μL)	0.07 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	47.2 ± 0.2	47.4 ± 0.2	46.8 ± 0.2	46.9 ± 0.1	48.0 ± 0.3
Mean cell hemoglobin (pg)	15.8 ± 0.1	15.6 ± 0.1	15.5 ± 0.1	15.6 ± 0.1	15.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.3 ± 0.2	33.0 ± 0.3	33.1 ± 0.1	33.1 ± 0.2	32.5 ± 0.4
Platelets (10^3/μL)	662.7 ± 40.3	608.1 ± 28.1	705.5 ± 61.1	606.6 ± 37.1	696.0 ± 61.1
Leukocytes (10^3/μL))	5.02 ± 0.29	4.55 ± 0.21	4.27 ± 0.38	4.49 ± 0.49	4.13 ± 0.51
Segmented neutrophils (10^3/μL)	0.79 ± 0.11	0.62 ± 0.11	0.56 ± 0.15	0.64 ± 0.06	0.58 ± 0.11
Lymphocytes (10^3/μL)	4.04 ± 0.26	3.75 ± 0.14	3.53 ± 0.26	3.65 ± 0.42	3.45 ± 0.43
Monocytes (10^3/μL)	0.05 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.07 ± 0.04	0.04 ± 0.02
Eosinophils (10^3/μL)	0.14 ± 0.03	0.13 ± 0.03	0.13 ± 0.02	0.12 ± 0.02	0.07 ± 0.03

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data were available for the 125 mg/kg males and females due to 100% mortality.
[b] No standard error was calculated because fewer than two measurements were available.

TABLE C5
Hematology Data for Mice in the 13-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg
Male						
n	10	10	10	10	10	10
Hematocrit (%)	49.8 ± 0.2	48.9 ± 0.4	50.0 ± 0.6	49.4 ± 0.5	51.0 ± 0.6	49.7 ± 0.5
Hemoglobin (g/dL)	15.9 ± 0.1	15.7 ± 0.1	16.0 ± 0.2	16.0 ± 0.2	16.3 ± 0.2	15.8 ± 0.1
Erythrocytes (10^6/μL)	10.43 ± 0.07	10.25 ± 0.10	10.44 ± 0.15	10.32 ± 0.09	10.66 ± 0.11	10.37 ± 0.09
Reticulocytes (10^6/μL)	1.20 ± 0.12	1.55 ± 0.14	1.22 ± 0.16	1.47 ± 0.13	1.57 ± 0.20	1.46 ± 0.11
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	47.8 ± 0.3	47.7 ± 0.2	47.8 ± 0.2	47.7 ± 0.2	47.8 ± 0.3	47.9 ± 0.2
Mean cell hemoglobin (pg)	15.2 ± 0.1	15.3 ± 0.1	15.3 ± 0.1	15.4 ± 0.1	15.3 ± 0.1	15.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.8 ± 0.1	32.1 ± 0.1	32.0 ± 0.1	32.3 ± 0.2	32.0 ± 0.3	31.8 ± 0.2
Platelets (10^3/μL)	977.4 ± 28.9	992.3 ± 15.8	1,002.2 ± 17.1	1,017.1 ± 22.5	1,061.4 ± 24.7	1,091.8 ± 20.6**
Leukocytes (10^3/μL)	5.20 ± 0.25	5.85 ± 0.41	4.82 ± 0.28	4.95 ± 0.26	4.83 ± 0.45	4.34 ± 0.19*
Segmented neutrophils (10^3/μL)	0.76 ± 0.08	0.82 ± 0.12	0.57 ± 0.09	0.69 ± 0.08	0.62 ± 0.09	0.59 ± 0.09
Lymphocytes (10^3/μL)	4.34 ± 0.22	4.94 ± 0.35	4.21 ± 0.24	4.20 ± 0.19	4.14 ± 0.38	3.67 ± 0.15*
Monocytes (10^3/μL)	0.03 ± 0.02	0.04 ± 0.03	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.01	0.03 ± 0.02
Eosinophils (10^3/μL)	0.07 ± 0.03	0.05 ± 0.02	0.02 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.01
Female						
n	10	10	10	10	10	9
Hematocrit (%)	46.8 ± 0.4	47.4 ± 0.5	47.4 ± 0.5	48.0 ± 0.5	48.3 ± 0.4	48.2 ± 0.7
Hemoglobin (g/dL)	15.2 ± 0.1	15.4 ± 0.1	15.6 ± 0.1	15.4 ± 0.1	15.6 ± 0.1	15.7 ± 0.2
Erythrocytes (10^6/μL)	9.88 ± 0.09	9.91 ± 0.09	9.95 ± 0.10	10.11 ± 0.12	10.19 ± 0.09	10.14 ± 0.16
Reticulocytes (10^6/μL)	1.21 ± 0.12	1.08 ± 0.12	1.22 ± 0.16	1.33 ± 0.18	1.17 ± 0.14	1.21 ± 0.06
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	47.6 ± 0.2	47.9 ± 0.2	47.8 ± 0.1	47.6 ± 0.2	47.4 ± 0.2	47.7 ± 0.2
Mean cell hemoglobin (pg)	15.4 ± 0.1	15.6 ± 0.1	15.7 ± 0.1	15.3 ± 0.1	15.3 ± 0.1	15.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.5 ± 0.2	32.5 ± 0.2	32.9 ± 0.3	32.2 ± 0.1	32.4 ± 0.2	32.6 ± 0.2
Platelets (10^3/μL)	843.8 ± 14.8	914.3 ± 18.3**	888.5 ± 26.8*	947.1 ± 18.3**	944.8 ± 21.9**	942.9 ± 34.2**
Leukocytes (10^3/μL)	4.97 ± 0.22	4.86 ± 0.22	4.71 ± 0.27	4.50 ± 0.47	4.52 ± 0.24	4.27 ± 0.20
Segmented neutrophils (10^3/μL)	0.63 ± 0.10	0.50 ± 0.09	0.52 ± 0.04	0.54 ± 0.08[b]	0.75 ± 0.16	0.55 ± 0.09
Lymphocytes (10^3/μL)	4.23 ± 0.15	4.29 ± 0.18	4.10 ± 0.25	3.70 ± 0.31	3.70 ± 0.15	3.63 ± 0.19
Monocytes (10^3/μL)	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.01
Eosinophils (10^3/μL)	0.08 ± 0.02	0.06 ± 0.02	0.07 ± 0.01	0.09 ± 0.03	0.04 ± 0.01	0.06 ± 0.02

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

TABLE C6
Hematology Data for Mice in the 14-Week Gavage Study of Acrolein[a]

	Vehicle Control	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Male					
n	9	9	10	9	9
Hematocrit (%)	53.0 ± 0.9	50.5 ± 0.7	52.8 ± 1.2	52.1 ± 1.1	60.0 ± 1.8
Hemoglobin (g/dL)	16.9 ± 0.3	16.2 ± 0.1	16.9 ± 0.4	16.7 ± 0.3	18.7 ± 0.5*
Erythrocytes (10^6/μL)	11.2 ± 0.2	10.6 ± 0.2	11.1 ± 0.3	11.0 ± 0.2	12.5 ± 0.4
Reticulocytes (10^6/μL))	0.13 ± 0.02	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.02	0.13 ± 0.02
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	47.4 ± 0.2	47.4 ± 0.2	47.4 ± 0.3	47.4 ± 0.2	48.0 ± 0.2
Mean cell hemoglobin (pg)	15.1 ± 0.0	15.2 ± 0.1	15.2 ± 0.1	15.2 ± 0.1	15.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.9 ± 0.1	32.0 ± 0.2	32.1 ± 0.1	32.2 ± 0.3	31.3 ± 0.3
Platelets (10^3/μL)	700.3 ± 41.1	808.6 ± 40.1	745.6 ± 34.9	821.3 ± 39.8	1,052.1 ± 56.0**
Leukocytes (10^3/μL)	4.54 ± 0.41	5.00 ± 0.42	5.16 ± 0.29	5.51 ± 0.41	4.11 ± 0.22
Segmented neutrophils (10^3/μL)	0.57 ± 0.10	0.76 ± 0.12	0.77 ± 0.11	0.74 ± 0.14	0.88 ± 0.13
Lymphocytes (10^3/μL)	3.92 ± 0.31	4.12 ± 0.30	4.30 ± 0.22	4.68 ± 0.38	3.16 ± 0.12
Monocytes (10^3/μL)	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Eosinophils (10^3/μL)	0.04 ± 0.02	0.10 ± 0.04	0.08 ± 0.02	0.08 ± 0.01	0.06 ± 0.01
Female					
n	9	10	10	9	7
Hematocrit (%)	47.4 ± 0.6	47.8 ± 0.8	49.5 ± 0.7*	50.0 ± 0.4**	53.4 ± 1.4**
Hemoglobin (g/dL)	15.6 ± 0.1	15.6 ± 0.2	16.0 ± 0.2	16.3 ± 0.2**	17.2 ± 0.4**
Erythrocytes (10^6/μL)	10.0 ± 0.1	10.1 ± 0.2	10.5 ± 0.1*	10.6 ± 0.1**	11.1 ± 0.3**
Reticulocytes (10^6/μL)	0.11 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	47.6 ± 0.2	47.2 ± 0.1	47.1 ± 0.2	47.4 ± 0.2	48.1 ± 0.1
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.4 ± 0.1	15.2 ± 0.1*	15.5 ± 0.1	15.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.8 ± 0.2	32.7 ± 0.2	32.3 ± 0.2	32.6 ± 0.1	32.2 ± 0.3
Platelets (10^3/μL)	678.4 ± 30.0	678.4 ± 12.5	659.7 ± 28.4	691.8 ± 37.2	821.7 ± 60.3
Leukocytes (10^3/μL)	4.78 ± 0.16	4.90 ± 0.24	4.30 ± 0.26	4.26 ± 0.19	3.90 ± 0.43
Segmented neutrophils (10^3/μL))	0.53 ± 0.07	0.74 ± 0.08	0.46 ± 0.05	0.56 ± 0.07	0.51 ± 0.09
Lymphocytes (10^3/μL)	4.13 ± 0.17	4.04 ± 0.20	3.77 ± 0.24	3.62 ± 0.17	3.34 ± 0.36
Monocytes (10^3/μL)	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Eosinophils (10^3/μL)	0.09 ± 0.03	0.10 ± 0.02	0.06 ± 0.02	0.07 ± 0.01	0.05 ± 0.02

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Shirley's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data. No data were available for the 20 mg/kg males and females due to 100% mortality.

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

TABLE D1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg
Male					
n	10	10	10	10	10
Necropsy body wt	349 ± 5	334 ± 6	329 ± 5*	341 ± 4	326 ± 7*
Heart					
Absolute	0.982 ± 0.018	0.993 ± 0.018	0.973 ± 0.029	0.942 ± 0.015	0.939 ± 0.021
Relative	2.82 ± 0.07	2.97 ± 0.03	2.95 ± 0.05	2.77 ± 0.05	2.88 ± 0.05
R. Kidney					
Absolute	1.011 ± 0.014	1.002 ± 0.025	1.021 ± 0.026	0.983 ± 0.024	1.021 ± 0.023
Relative	2.90 ± 0.04	3.00 ± 0.05	3.10 ± 0.04*	2.89 ± 0.07	3.13 ± 0.04**
Liver					
Absolute	12.107 ± 0.271	11.760 ± 0.236	11.930 ± 0.420	12.201 ± 0.254	11.999 ± 0.353
Relative	34.73 ± 0.68	35.22 ± 0.63	36.24 ± 0.97	35.83 ± 0.56	36.80 ± 0.68
Lung					
Absolute	1.510 ± 0.051	1.500 ± 0.035	1.476 ± 0.020	1.571 ± 0.059	1.492 ± 0.057
Relative	4.33 ± 0.12	4.49 ± 0.09	4.50 ± 0.08	4.61 ± 0.16	4.58 ± 0.14
R. Testis					
Absolute	1.445 ± 0.019	1.455 ± 0.018	1.515 ± 0.083	1.410 ± 0.017	1.424 ± 0.023
Relative	4.15 ± 0.06	4.36 ± 0.07	4.61 ± 0.24*	4.14 ± 0.05	4.38 ± 0.07
Thymus					
Absolute	0.319 ± 0.019	0.280 ± 0.013	0.282 ± 0.012	0.328 ± 0.013	0.273 ± 0.015
Relative	0.92 ± 0.05	0.84 ± 0.04	0.86 ± 0.03	0.96 ± 0.03	0.84 ± 0.03
Female					
n	9	10	10	10	10
Necropsy body wt	194 ± 3	191 ± 3	194 ± 4	192 ± 3	191 ± 3
Heart					
Absolute	0.640 ± 0.009	0.681 ± 0.041	0.634 ± 0.012	0.642 ± 0.011	0.624 ± 0.013
Relative	3.30 ± 0.04	3.57 ± 0.21	3.27 ± 0.06	3.35 ± 0.08	3.26 ± 0.03
R. Kidney					
Absolute	0.641 ± 0.016	0.629 ± 0.018	0.627 ± 0.017	0.631 ± 0.006	0.651 ± 0.013
Relative	3.30 ± 0.06	3.29 ± 0.07	3.22 ± 0.05	3.29 ± 0.05	3.40 ± 0.06
Liver					
Absolute	6.418 ± 0.144	6.335 ± 0.128	6.209 ± 0.167	6.285 ± 0.117	7.372 ± 0.241**
Relative	33.06 ± 0.41	33.20 ± 0.43	31.94 ± 0.59	32.79 ± 0.69	38.44 ± 0.76**
Lung					
Absolute	1.030 ± 0.028	1.062 ± 0.019	1.063 ± 0.022	1.062 ± 0.035	1.062 ± 0.030
Relative	5.32 ± 0.15	5.57 ± 0.11	5.48 ± 0.11	5.53 ± 0.15	5.55 ± 0.15
Thymus					
Absolute	0.234 ± 0.015	0.254 ± 0.015	0.247 ± 0.009	0.245 ± 0.007	0.242 ± 0.010
Relative	1.20 ± 0.08	1.33 ± 0.07	1.27 ± 0.03	1.28 ± 0.03	1.26 ± 0.05

* 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). No data were available for the 100 mg/kg males or females due to 100% mortality.

TABLE D2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	348 ± 7	337 ± 4	335 ± 6	348 ± 6	346 ± 6	341 ± 7
Heart						
Absolute	1.041 ± 0.027	1.075 ± 0.033	1.024 ± 0.030	1.071 ± 0.021	1.066 ± 0.022	1.027 ± 0.023
Relative	2.99 ± 0.06	3.18 ± 0.07	3.06 ± 0.07	3.09 ± 0.08	3.08 ± 0.05	3.01 ± 0.04
R. Kidney						
Absolute	1.080 ± 0.025	1.044 ± 0.028	1.055 ± 0.020	1.091 ± 0.014	1.065 ± 0.023	1.080 ± 0.021
Relative	3.11 ± 0.05	3.09 ± 0.06	3.16 ± 0.06	3.14 ± 0.04	3.08 ± 0.04	3.17 ± 0.04
Liver						
Absolute	11.654 ± 0.312	11.950 ± 0.307	11.330 ± 0.256	12.312 ± 0.339	12.350 ± 0.288	12.635 ± 0.251*
Relative	33.45 ± 0.31	35.40 ± 0.62	33.85 ± 0.34	35.40 ± 0.65**	35.67 ± 0.31**	37.04 ± 0.34**
Lung						
Absolute	1.632 ± 0.069	1.580 ± 0.022	1.665 ± 0.046	1.594 ± 0.077	1.715 ± 0.067	1.526 ± 0.075
Relative	4.70 ± 0.21	4.69 ± 0.09	4.99 ± 0.18	4.59 ± 0.22	4.97 ± 0.21	4.46 ± 0.17
R. Testis						
Absolute	1.426 ± 0.024	1.401 ± 0.037	1.414 ± 0.018	1.456 ± 0.018	1.469 ± 0.023	1.434 ± 0.027
Relative	4.11 ± 0.08	4.16 ± 0.12	4.24 ± 0.09	4.20 ± 0.06	4.25 ± 0.06	4.21 ± 0.06
Thymus						
Absolute	0.286 ± 0.016	0.266 ± 0.010	0.268 ± 0.014	0.283 ± 0.018	0.285 ± 0.014	0.266 ± 0.010
Relative	0.82 ± 0.04	0.79 ± 0.03	0.80 ± 0.04	0.81 ± 0.04	0.82 ± 0.03	0.78 ± 0.03
Female						
n	10	10	10	9	10	10
Necropsy body wt	207 ± 5	203 ± 4	201 ± 4	205 ± 4	209 ± 5	205 ± 3
Heart						
Absolute	0.734 ± 0.016	0.706 ± 0.017	0.688 ± 0.015	0.767 ± 0.025	0.763 ± 0.016	0.713 ± 0.010
Relative	3.56 ± 0.07	3.49 ± 0.07	3.44 ± 0.05	3.75 ± 0.13	3.66 ± 0.07	3.48 ± 0.05
R. Kidney						
Absolute	0.676 ± 0.022	0.667 ± 0.017	0.663 ± 0.015	0.698 ± 0.008	0.694 ± 0.017	0.679 ± 0.017
Relative	3.27 ± 0.07	3.29 ± 0.06	3.31 ± 0.05	3.41 ± 0.05	3.33 ± 0.06	3.31 ± 0.05
Liver						
Absolute	7.008 ± 0.180	6.685 ± 0.109	6.813 ± 0.208	6.866 ± 0.148	6.943 ± 0.221	7.428 ± 0.160
Relative	33.91 ± 0.39	33.00 ± 0.34	33.96 ± 0.61	33.53 ± 0.40	33.27 ± 0.66	36.19 ± 0.38**
Lung						
Absolute	1.289 ± 0.070	1.257 ± 0.056	1.249 ± 0.039	1.224 ± 0.058	1.171 ± 0.031	1.145 ± 0.044
Relative	6.21 ± 0.24	6.19 ± 0.22	6.23 ± 0.11	5.98 ± 0.25	5.64 ± 0.19	5.60 ± 0.26
Thymus						
Absolute	0.265 ± 0.009	0.253 ± 0.008	0.243 ± 0.005	0.252 ± 0.009	0.257 ± 0.013	0.231 ± 0.011
Relative	1.29 ± 0.05	1.25 ± 0.03	1.21 ± 0.02	1.23 ± 0.05	1.24 ± 0.07	1.13 ± 0.05

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

TABLE D3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Gavage Study of Acrolein[a]

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Male						
n	10	10	10	8	8	1[b]
Necropsy body wt	350 ± 7	347 ± 4	350 ± 6	362 ± 7	341 ± 8	281
Heart						
Absolute	1.064 ± 0.022	1.024 ± 0.009	1.033 ± 0.025	1.081 ± 0.046	1.011 ± 0.012	0.821
Relative	3.04 ± 0.05	2.96 ± 0.04	2.95 ± 0.08	2.99 ± 0.09	2.98 ± 0.06	2.93
R. Kidney						
Absolute	1.061 ± 0.017	1.063 ± 0.019	1.094 ± 0.038	1.128 ± 0.018	1.082 ± 0.022	0.916
Relative	3.03 ± 0.04	3.07 ± 0.04	3.12 ± 0.08	3.13 ± 0.05	3.18 ± 0.07	3.27
Liver						
Absolute	12.228 ± 0.332	12.242 ± 0.271	12.531 ± 0.514	13.481 ± 0.375	12.916 ± 0.317	11.820
Relative	34.90 ± 0.59	35.32 ± 0.71	35.73 ± 1.12	37.27 ± 0.46*	37.95 ± 0.46**	42.14
Lung						
Absolute	1.852 ± 0.079	1.803 ± 0.120	1.850 ± 0.124	1.775 ± 0.111	1.853 ± 0.079	1.201
Relative	5.30 ± 0.22	5.23 ± 0.39	5.28 ± 0.34	4.93 ± 0.34	5.47 ± 0.27	4.28
R. Testis						
Absolute	1.457 ± 0.017	1.464 ± 0.022	1.485 ± 0.022	1.496 ± 0.015	1.452 ± 0.027	1.319
Relative	4.17 ± 0.07	4.22 ± 0.06	4.25 ± 0.09	4.15 ± 0.08	4.28 ± 0.09	4.70
Thymus						
Absolute	0.316 ± 0.017	0.301 ± 0.018	0.327 ± 0.010	0.317 ± 0.008	0.289 ± 0.019	0.235
Relative	0.90 ± 0.05	0.86 ± 0.05	0.93 ± 0.03	0.88 ± 0.03	0.85 ± 0.04	0.84
Female						
n	10	10	9	8	9	2
Necropsy body wt	195 ± 4	198 ± 3	199 ± 3	192 ± 3	189 ± 3	175 ± 2*
Heart						
Absolute	0.703 ± 0.012	0.716 ± 0.015	0.714 ± 0.024	0.714 ± 0.031	0.638 ± 0.015*	0.602 ± 0.017*
Relative	3.62 ± 0.08	3.63 ± 0.10	3.60 ± 0.10	3.71 ± 0.13	3.38 ± 0.07	3.45 ± 0.14
R. Kidney						
Absolute	0.646 ± 0.021	0.657 ± 0.013	0.670 ± 0.015	0.650 ± 0.011	0.635 ± 0.015	0.558 ± 0.013*
Relative	3.32 ± 0.06	3.32 ± 0.05	3.37 ± 0.06	3.38 ± 0.04	3.36 ± 0.05	3.20 ± 0.03
Liver						
Absolute	6.800 ± 0.188	6.614 ± 0.095	6.594 ± 0.161	6.975 ± 0.145	7.313 ± 0.199*	7.690 ± 0.429*
Relative	34.94 ± 0.56	33.48 ± 0.39	33.20 ± 0.50	36.28 ± 0.65	38.76 ± 0.90**	44.05 ± 1.86**
Lung						
Absolute	1.136 ± 0.040	1.207 ± 0.043	1.245 ± 0.066	1.172 ± 0.046	1.136 ± 0.029	1.214 ± 0.018
Relative	5.85 ± 0.20	6.11 ± 0.20	6.27 ± 0.32	6.10 ± 0.25	6.03 ± 0.16	6.96 ± 0.19
Thymus						
Absolute	0.234 ± 0.008	0.235 ± 0.007	0.261 ± 0.008	0.239 ± 0.006	0.228 ± 0.011	0.165 ± 0.028**
Relative	1.21 ± 0.05	1.19 ± 0.04	1.31 ± 0.03	1.24 ± 0.03	1.21 ± 0.06	0.95 ± 0.17*

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] No standard error was calculated because less than two measurements were available.

TABLE D4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	8 mg/kg	16 mg/kg	32 mg/kg	62.5 mg/kg
Male					
n	9	7	10	9	2
Necropsy body wt	37.2 ± 1.1	39.0 ± 0.8	37.2 ± 0.8	36.9 ± 1.0	37.0 ± 0.5
Heart					
Absolute	0.156 ± 0.003	0.159 ± 0.002	0.158 ± 0.004	0.158 ± 0.003	0.155 ± 0.012
Relative	4.22 ± 0.14	4.08 ± 0.04	4.25 ± 0.07	4.29 ± 0.11	4.19 ± 0.36
R. Kidney					
Absolute	0.307 ± 0.007	0.311 ± 0.008	0.307 ± 0.007	0.301 ± 0.006	0.318 ± 0.022
Relative	8.29 ± 0.19	7.99 ± 0.12	8.28 ± 0.22	8.19 ± 0.21	8.61 ± 0.70
Liver					
Absolute	1.711 ± 0.044	1.744 ± 0.030	1.678 ± 0.038	1.611 ± 0.051	1.640 ± 0.051
Relative	46.06 ± 0.33	44.78 ± 0.32	45.13 ± 0.66	43.59 ± 0.63**	44.37 ± 0.84
Lung					
Absolute	0.275 ± 0.014	0.319 ± 0.022	0.310 ± 0.015	0.276 ± 0.017	0.298 ± 0.001
Relative	7.45 ± 0.43	8.22 ± 0.60	8.34 ± 0.38	7.49 ± 0.44	8.07 ± 0.13
R. Testis					
Absolute	0.119 ± 0.003	0.123 ± 0.004	0.116 ± 0.003	0.123 ± 0.002	0.121 ± 0.002
Relative	3.22 ± 0.10	3.17 ± 0.10	3.13 ± 0.06	3.34 ± 0.09	3.26 ± 0.08
Thymus					
Absolute	0.055 ± 0.004	0.050 ± 0.003	0.046 ± 0.002	0.053 ± 0.002	0.047 ± 0.010
Relative	1.47 ± 0.08	1.29 ± 0.06	1.23 ± 0.04*	1.43 ± 0.04	1.26 ± 0.24
Female					
n	9	8	6	7	6
Necropsy body wt	31.5 ± 0.6	33.3 ± 0.6	32.0 ± 1.4	30.2 ± 1.0	29.1 ± 1.1
Heart					
Absolute	0.130 ± 0.005	0.126 ± 0.003	0.127 ± 0.002	0.127 ± 0.004	0.134 ± 0.008
Relative	4.13 ± 0.15	3.78 ± 0.05	3.99 ± 0.10	4.24 ± 0.21	4.61 ± 0.20
R. Kidney					
Absolute	0.177 ± 0.003	0.184 ± 0.003	0.186 ± 0.007	0.172 ± 0.004	0.175 ± 0.008
Relative	5.65 ± 0.15	5.54 ± 0.13	5.83 ± 0.09	5.70 ± 0.15	6.03 ± 0.20
Liver					
Absolute	1.301 ± 0.036	1.329 ± 0.034	1.372 ± 0.042	1.259 ± 0.047	1.346 ± 0.080
Relative	41.33 ± 0.83	39.91 ± 0.65	43.05 ± 1.45	41.65 ± 0.93	46.11 ± 1.45**
Lung					
Absolute	0.250 ± 0.014	0.269 ± 0.015	0.256 ± 0.019	0.265 ± 0.020	0.277 ± 0.021
Relative	7.99 ± 0.51	8.12 ± 0.50	8.04 ± 0.66	8.85 ± 0.82	9.47 ± 0.51
Thymus					
Absolute	0.055 ± 0.003	0.051 ± 0.004	0.053 ± 0.003	0.050 ± 0.005	0.052 ± 0.004
Relative	1.75 ± 0.08	1.52 ± 0.11	1.66 ± 0.12	1.66 ± 0.16	1.77 ± 0.14

* 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). No data were available for the 125 mg/kg males or females due to 100% mortality.

TABLE D5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	42.4 ± 1.2	40.9 ± 1.3	42.0 ± 0.8	41.3 ± 0.9	40.6 ± 1.1	39.3 ± 0.6
Heart						
Absolute	0.172 ± 0.008	0.175 ± 0.006	0.180 ± 0.007	0.167 ± 0.005	0.166 ± 0.006	0.159 ± 0.005
Relative	4.05 ± 0.13	4.28 ± 0.11	4.31 ± 0.21	4.04 ± 0.10	4.10 ± 0.11	4.04 ± 0.10
R. Kidney						
Absolute	0.320 ± 0.014	0.316 ± 0.006	0.316 ± 0.010	0.324 ± 0.008	0.315 ± 0.010	0.313 ± 0.007
Relative	7.55 ± 0.24	7.77 ± 0.10	7.53 ± 0.23	7.86 ± 0.21	7.79 ± 0.22	7.97 ± 0.14
Liver						
Absolute	1.783 ± 0.065	1.694 ± 0.070	1.784 ± 0.053	1.795 ± 0.042	1.773 ± 0.103	1.826 ± 0.040
Relative	42.01 ± 0.92	41.44 ± 0.94	42.47 ± 1.04	43.46 ± 0.54	43.45 ± 1.31	46.54 ± 0.95**
Lung						
Absolute	0.226 ± 0.009	0.218 ± 0.010	0.230 ± 0.012	0.227 ± 0.012	0.242 ± 0.012	0.247 ± 0.010
Relative	5.34 ± 0.17	5.38 ± 0.27	5.46 ± 0.24	5.54 ± 0.33	5.97 ± 0.26	6.29 ± 0.23*
R. Testis						
Absolute	0.128 ± 0.004	0.119 ± 0.004	0.123 ± 0.003	0.125 ± 0.003	0.124 ± 0.004	0.110 ± 0.010
Relative	3.01 ± 0.06	2.91 ± 0.07	2.94 ± 0.06	3.03 ± 0.08	3.06 ± 0.08	2.81 ± 0.24
Thymus						
Absolute	0.055 ± 0.004	0.056 ± 0.005	0.051 ± 0.003	0.053 ± 0.003	0.054 ± 0.003	0.046 ± 0.002
Relative	1.29 ± 0.08	1.35 ± 0.10	1.20 ± 0.04	1.28 ± 0.08	1.33 ± 0.08	1.18 ± 0.06
Female						
n	10	10	10	10	10	9
Necropsy body wt	32.7 ± 1.2	34.7 ± 1.4	36.7 ± 1.2	34.1 ± 1.1	33.2 ± 1.2	32.2 ± 1.1
Heart						
Absolute	0.135 ± 0.004	0.138 ± 0.004	0.136 ± 0.003	0.133 ± 0.003	0.133 ± 0.003	0.133 ± 0.005
Relative	4.16 ± 0.13	4.04 ± 0.22	3.75 ± 0.15	3.95 ± 0.17	4.06 ± 0.15	4.15 ± 0.17
R. Kidney						
Absolute	0.167 ± 0.004	0.169 ± 0.003	0.186 ± 0.004**	0.173 ± 0.004	0.176 ± 0.004	0.181 ± 0.005
Relative	5.15 ± 0.17	4.94 ± 0.19	5.09 ± 0.12	5.11 ± 0.20	5.34 ± 0.17	5.63 ± 0.17
Liver						
Absolute	1.304 ± 0.036	1.363 ± 0.045	1.447 ± 0.026	1.305 ± 0.031	1.331 ± 0.052	1.442 ± 0.047
Relative	40.07 ± 1.00	39.43 ± 0.57	39.66 ± 0.92	38.47 ± 1.01	40.34 ± 1.45	44.77 ± 0.58**
Lung						
Absolute	0.201 ± 0.006	0.195 ± 0.006	0.224 ± 0.007	0.207 ± 0.007	0.211 ± 0.004	0.220 ± 0.013
Relative	6.21 ± 0.28	5.65 ± 0.16	6.17 ± 0.32	6.10 ± 0.26	6.42 ± 0.22	6.88 ± 0.43
Thymus						
Absolute	0.051 ± 0.003	0.048 ± 0.004	0.053 ± 0.004	0.054 ± 0.003	0.049 ± 0.002	0.054 ± 0.003
Relative	1.57 ± 0.10	1.39 ± 0.10	1.45 ± 0.10	1.58 ± 0.09	1.46 ± 0.04	1.67 ± 0.11

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

TABLE D6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study of Acrolein[a]

	Vehicle Control	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
Male					
n	10	9	10	9	9
Necropsy body wt	37.1 ± 1.4	37.4 ± 1.0	39.2 ± 1.1	37.9 ± 0.5	36.1 ± 0.9
Heart					
Absolute	0.167 ± 0.005	0.179 ± 0.011	0.173 ± 0.007	0.166 ± 0.005	0.169 ± 0.006
Relative	4.55 ± 0.17	4.78 ± 0.23	4.44 ± 0.19	4.37 ± 0.13	4.70 ± 0.16
R. Kidney					
Absolute	0.310 ± 0.005	0.306 ± 0.008	0.324 ± 0.009	0.317 ± 0.004	0.316 ± 0.009
Relative	8.44 ± 0.31	8.22 ± 0.25	8.30 ± 0.18	8.38 ± 0.10	8.76 ± 0.15
Liver					
Absolute	1.710 ± 0.063	1.705 ± 0.076	1.781 ± 0.054	1.828 ± 0.029	1.969 ± 0.049**
Relative	46.12 ± 0.72	45.50 ± 0.93	45.50 ± 0.61	48.23 ± 0.64	54.75 ± 1.46**
Lung					
Absolute	0.347 ± 0.016	0.295 ± 0.013	0.321 ± 0.022	0.292 ± 0.018	0.315 ± 0.027
Relative	9.39 ± 0.43	7.92 ± 0.35	8.27 ± 0.62	7.70 ± 0.47	8.81 ± 0.83
R. Testis					
Absolute	0.117 ± 0.004	0.120 ± 0.003	0.115 ± 0.003	0.118 ± 0.002	0.116 ± 0.003
Relative	3.16 ± 0.09	3.22 ± 0.07	2.94 ± 0.06	3.10 ± 0.07	3.22 ± 0.12
Thymus					
Absolute	0.047 ± 0.002	0.045 ± 0.002	0.046 ± 0.003	0.049 ± 0.004	0.046 ± 0.004
Relative	1.28 ± 0.06	1.22 ± 0.07	1.18 ± 0.08	1.30 ± 0.12	1.27 ± 0.10
Female					
n	9	10	10	9	8
Necropsy body wt	31.4 ± 1.1	35.2 ± 1.6	32.7 ± 1.1	27.7 ± 0.9	29.0 ± 0.8
Heart					
Absolute	0.161 ± 0.010	0.147 ± 0.003	0.142 ± 0.004	0.149 ± 0.008	0.146 ± 0.009
Relative	5.15 ± 0.31	4.24 ± 0.18*	4.37 ± 0.14	5.40 ± 0.27	5.02 ± 0.24
R. Kidney					
Absolute	0.184 ± 0.008	0.201 ± 0.008	0.184 ± 0.003	0.174 ± 0.005	0.188 ± 0.005
Relative	5.87 ± 0.14	5.74 ± 0.13	5.68 ± 0.15	6.29 ± 0.14	6.49 ± 0.15**
Liver					
Absolute	1.281 ± 0.047	1.368 ± 0.048	1.328 ± 0.032	1.221 ± 0.038	1.405 ± 0.055
Relative	40.83 ± 0.71	39.14 ± 0.89	40.89 ± 1.24	44.10 ± 0.65*	48.47 ± 0.99**
Lung					
Absolute	0.259 ± 0.020	0.300 ± 0.013	0.291 ± 0.010	0.260 ± 0.014	0.290 ± 0.021
Relative	8.26 ± 0.59	8.69 ± 0.50	9.03 ± 0.50	9.38 ± 0.41	9.97 ± 0.57
Thymus					
Absolute	0.053 ± 0.003	0.061 ± 0.005	0.052 ± 0.002	0.047 ± 0.002	0.049 ± 0.003
Relative	1.69 ± 0.07	1.72 ± 0.07	1.60 ± 0.06	1.71 ± 0.06	1.69 ± 0.06

* 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). No data were available for the 20 mg/kg males or females due to 100% mortality.

APPENDIX E
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE E1
Summary of Reproductive Tissue Evaluations for Male Rats in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	12 mg/kg	25 mg/kg	50 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	349 ± 5	329 ± 5*	340 ± 4	326 ± 7*
L. cauda epididymis	0.1463 ± 0.0035	0.1410 ± 0.0059	0.1405 ± 0.0032	0.1476 ± 0.0046
L. epididymis	0.4483 ± 0.0053	0.4599 ± 0.0082	0.4524 ± 0.0038	0.4584 ± 0.0137
L. testis	1.4590 ± 0.0215	1.4923 ± 0.0244	1.4888 ± 0.0204	1.4858 ± 0.0312
Spermatid measurements				
Spermatid heads (10^7/g testis)	7.57 ± 0.25	7.46 ± 0.26	7.34 ± 0.28	7.83 ± 0.26
Spermatid heads (10^7/testis)	11.04 ± 0.38	11.12 ± 0.40	10.92 ± 0.44	11.61 ± 0.34
Spermatid count (mean/10^{-4} mL suspension)	55.18 ± 1.90	55.60 ± 1.99	54.60 ± 2.19	58.03 ± 1.68
Epididymal spermatozoal measurements				
Motility (%)	64.36 ± 1.50	62.65 ± 2.53	61.70 ± 2.28	62.35 ± 1.52
Concentration (10^6/g cauda epididymal tissue)	605 ± 41	509 ± 44[b]	544 ± 37	473 ± 31

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's 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).
[b] n=9

TABLE E2
Estrous Cycle Characterization for Female Rats in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	12 mg/kg	25 mg/kg	50 mg/kg
n	10	10	10	10
Necropsy body wt (g)	194 ± 3	194 ± 4	192 ± 3	191 ± 3
Estrous cycle length (days)	4.75 ± 0.20	4.56 ± 0.18[b]	4.40 ± 0.16	4.85 ± 0.27
Estrous stages (% of cycle)				
Diestrus	45.8	58.3	56.7	56.7
Proestrus	12.5	14.2	12.5	15.8
Estrus	30.8	20.8	24.2	23.3
Metestrus	10.8	6.7	6.7	4.2

[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 the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in one of 10 animals.

TABLE E3
Summary of Reproductive Tissue Evaluation for Male Rats in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	348 ± 6	348 ± 6	346 ± 6	341 ± 7
L. cauda epididymis	0.1513 ± 0.0028	0.1572 ± 0.0027	0.1517 ± 0.0028	0.1599 ± 0.0040
L. epididymis	0.4656 ± 0.0076	0.4492 ± 0.0053	0.4734 ± 0.0092	0.4584 ± 0.0094
L. testis	1.5036 ± 0.0266	1.5165 ± 0.0178	1.5042 ± 0.0243	1.4966 ± 0.0278
Spermatid measurements				
Spermatid heads (10^7/g testis)	7.65 ± 0.38	7.80 ± 0.28	8.01 ± 0.26	8.22 ± 0.13
Spermatid heads (10^7/testis)	11.52 ± 0.62	11.85 ± 0.46	12.01 ± 0.29	12.29 ± 0.23
Spermatid count (mean/10^{-4} mL suspension)	57.58 ± 3.12	59.23 ± 2.32	60.05 ± 1.44	61.43 ± 1.13
Epididymal spermatozoal measurements				
Motility (%)	67.59 ± 0.43	67.30 ± 0.70	66.50 ± 0.70	67.71 ± 0.66
Concentration (10^6/g cauda epididymal tissue)	508 ± 28	490 ± 31	515 ± 27	444 ± 28

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

TABLE E4
Estrous Cycle Characterization for Female Rats in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg
n	10	9	10	10
Necropsy body wt (g)	207 ± 4	205 ± 4	209 ± 5	205 ± 3
Estrous cycle length (days)	4.60 ± 0.12	4.56 ± 0.15	4.29 ± 0.21[b]	4.60 ± 0.52
Estrous stages[c] (% of cycle)				
Diestrus	38.3	42.6	55.8	60.0
Proestrus	13.3	19.4	15.0	11.7
Estrus	30.8	25.9	21.7	25.8
Metestrus	17.5	12.0	7.5	2.5

[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length).
[b] Estrous cycle was longer than 12 days or unclear in three of 10 animals.
[c] Evidence suggests that females in the 25 mg/kg group differ significantly (Wilk's Criterion, P=0.0009) from the vehicle control females in the relative length of time spent in the estrous stages. Dosed females spent more time in diestrus and less time in metestrus than vehicle control females.

TABLE E5
Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	8 mg/kg	16 mg/kg	32 mg/kg
n	9	7	10	9
Weights (g)				
Necropsy body wt	37.2 ± 1.1	39.0 ± 0.8	37.2 ± 0.8	36.9 ± 1.0
L. cauda epididymis	0.0171 ± 0.0008	0.0197 ± 0.0008	0.0178 ± 0.0008	0.0184 ± 0.0007
L. epididymis	0.0539 ± 0.0022	0.0523 ± 0.0017	0.0531 ± 0.0014	0.0518 ± 0.0015
L. testis	0.1159 ± 0.0034	0.1306 ± 0.0085	0.1125 ± 0.0028	0.1232 ± 0.0041
Spermatid measurements				
Spermatid heads (10^7/g testis)	16.31 ± 0.56	13.95 ± 1.23	16.22 ± 0.68	15.18 ± 0.90
Spermatid heads (10^7/testis)	1.89 ± 0.09	1.78 ± 0.12	1.83 ± 0.09	1.85 ± 0.08
Spermatid count (mean/10^{-4} mL suspension)	59.14 ± 2.78	55.64 ± 3.89	57.03 ± 2.77	57.75 ± 2.40
Epididymal spermatozoal measurements				
Motility (%)	63.19 ± 0.79	60.23 ± 1.79	63.59 ± 1.04	60.98 ± 0.83
Concentration (10^6/g cauda epididymal tissue)	911 ± 69	850 ± 79	825 ± 73	973 ± 83

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

TABLE E6
Estrous Cycle Characterization for Female Mice in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	16 mg/kg	32 mg/kg	62.5 mg/kg
n	9	6	7	6
Necropsy body wt (g)	31.5 ± 0.6	32.0 ± 1.4	30.2 ± 1.0	29.1 ± 1.1
Estrous cycle length (days)	4.00 ± 0.00	5.92 ± 1.02*	5.92 ± 0.95*[b]	4.33 ± 0.25
Estrous stages (% of cycle)				
Diestrus	34.3	44.4	50.0	30.6
Proestrus	16.7	16.7	13.1	16.7
Estrus	26.9	22.2	21.4	30.6
Metestrus	22.2	16.7	15.5	22.2

* Significantly different ($P \leq 0.05$) from the vehicle control group 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 body weights are not significant by Dunnett's test. By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in one of seven animals.

TABLE E7
Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	12 mg/kg	25 mg/kg	50 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	42.4 ± 1.2	41.3 ± 0.9	40.6 ± 1.1	39.3 ± 0.6
L. cauda epididymis	0.0122 ± 0.0004	0.0139 ± 0.0010	0.0128 ± 0.0007	0.0119 ± 0.0005
L. epididymis	0.0371 ± 0.0008	0.0390 ± 0.0011	0.0384 ± 0.0009	0.0348 ± 0.0010
L. testis	0.1173 ± 0.0027	0.1224 ± 0.0022	0.1182 ± 0.0030	0.1086 ± 0.0076
Spermatid measurements				
Spermatid heads (10^7/g testis)	14.60 ± 0.69	15.35 ± 0.81	13.58 ± 0.89	14.46 ± 1.10
Spermatid heads (10^7/testis)	1.70 ± 0.07	1.88 ± 0.10	1.60 ± 0.11	1.62 ± 0.17
Spermatid count (10^{-4} mL suspension)	53.20 ± 2.06	58.75 ± 3.15	49.95 ± 3.31	50.48 ± 5.23
Epididymal spermatozoal measurements				
Motility (%)	66.93 ± 0.53	67.11 ± 0.89	68.09 ± 0.70	67.60 ± 0.57
Concentration (10^6/g cauda epididymal tissue)	1,079 ± 43	935 ± 96	1,026 ± 101	931 ± 111

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

TABLE E8
Estrous Cycle Characterization for Female Mice in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	12 mg/kg	25 mg/kg	50 mg/kg
n	10	10	10	9
Necropsy body wt (g)	32.7 ± 1.2	34.1 ± 1.1	33.2 ± 1.2	32.2 ± 1.1
Estrous cycle length (days)	4.86 ± 0.70[b]	5.33 ± 0.80[c]	4.28 ± 0.15[c]	4.21 ± 0.15[d]
Estrous stages (% of cycle)				
Diestrus	58.3	47.5	37.5	50.0
Proestrus	9.2	16.7	15.8	13.9
Estrus	17.5	19.2	23.3	19.4
Metestrus	15.0	16.7	23.3	16.7

[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 the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in three of 10 animals.
[c] Estrous cycle was longer than 12 days or unclear in one of 10 animals.
[d] Estrous cycle was longer than 12 days or unclear in two of nine animals.

APPENDIX F
3-HYDROXYPROPYL MERCAPTURIC ACID CONCENTRATIONS

TABLE F1
Urine 3-Hydroxypropyl Mercapturic Acid Concentrations for Rats in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg
n	10	10	10	10	10
Male					
1st dose	—[b]	17.66 ± 1.10**	51.83 ± 5.28**	126.09 ± 8.08**	355.10 ± 15.76**
45th dose	—	57.25 ± 2.74**	104.43 ± 8.34**	260.00 ± 18.61**	479.70 ± 33.78**
Female					
1st dose	—	18.68 ± 1.91**	63.87 ± 3.77**	147.94 ± 17.42**	335.20 ± 33.13**
45th dose	—	33.33 ± 2.56**	73.60 ± 4.17**	162.92 ± 17.88**	380.40 ± 36.09**

** Significantly different (P≤0.01) from the vehicle control group by Shirley's test
[a] Data are presented as µg/mL (mean ± standard error). No data were available for the 100 mg/kg males or females due to 100% mortality.
[b] Below the limit of detection (1.30 µg/mL)

TABLE F2
Urine 3-Hydroxypropyl Mercapturic Acid Concentrations for Rats in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg
n	10	10	10	10	10	10
Male						
1st dose	—[b]	13.42 ± 0.63**	22.33 ± 0.96**	54.17 ± 3.23**	103.25 ± 6.00**	239.20 ± 18.73**
45th dose	—	19.59 ± 1.10**	37.38 ± 2.68**	77.74 ± 4.81**	140.10 ± 17.59**[c]	354.40 ± 29.12**
Female						
1st dose	—	13.08 ± 0.75**	21.03 ± 0.82**[c]	44.47 ± 2.39**	100.22 ± 7.07**	249.13 ± 24.90**
45th dose	—	16.91 ± 1.41**	36.83 ± 2.14**	93.58 ± 7.12**[c]	183.40 ± 18.49**	453.80 ± 26.66**

** Significantly different (P≤0.01) from the vehicle control group by Shirley's test
[a] Data are presented as µg/mL (mean ± standard error). No data were available for the 20 mg/kg males or females due to 100% mortality.
[b] Below the limit of detection (1.30 µg/mL)
[c] n=9

TABLE F3
Urine 3-Hydroxypropyl Mercapturic Acid Concentrations for Rats in the 14-Week Gavage Study of Acrolein[a]

	Vehicle Control	0.75 mg/kg	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
n	10	10	10	10	10	10
Male						
1st dose	—[b]	13.82 ± 0.94**	18.41 ± 0.80**	32.64 ± 1.82**	61.65 ± 3.39**	63.85 ± 9.45**
45th dose	—	18.56 ± 1.61**	24.07 ± 1.48**	42.80 ± 3.02**[c]	69.61 ± 3.51**[c]	44.30 ±18.50**[d]
Female						
1st dose	—	12.60 ± 1.22**[c]	17.08 ± 1.39**	31.81 ± 2.46**	52.17 ± 3.68**	73.92 ± 8.15**
45th dose	—	11.09 ± 0.55**	19.34 ± 1.82**[e]	26.56 ± 2.90**[c]	49.22 ± 7.15**[e]	98.20[f]

** Significantly different (P≤0.01) from the vehicle control group by Shirley's test
[a] Data are presented as µg/mL (mean ± standard error). No data were available for the 100 mg/kg males and females due to 100% mortality.
[b] Below the limit of detection (1.30 µg/mL)
[c] n=8
[d] n=2
[e] n=9
[f] n=1; no standard error was calculated because less than two measurements were available.

TABLE F4
Urine 3-Hydroxypropyl Mercapturic Acid Concentrations for Mice in the 14-Week Gavage Study of Allyl Acetate[a]

	Vehicle Control	8 mg/kg	16 mg/kg	32 mg/kg	62.5 mg/kg
n	2	2	2	2	2
Male					
1st dose	11.24 ± 2.86	29.55 ± 5.15	84.25 ± 0.55	88.65 ± 7.05	108.20 ± 27.80
45th dose	17.25 ± 3.95	98.05 ± 19.95	171.00 ± 9.00	232.00 ± 93.00	157.50 ± 33.50
Female					
1st dose	—[b,c]	16.55 ± 1.95**	26.75 ± 1.45**	63.00 ± 0.00**	145.00 ± 15.00**
45th dose	—[c]	26.65 ± 3.15**	46.00 ± 14.00**	57.60 ± 2.10**	172.50 ± 0.50**

** Significantly different (P≤0.01) from the vehicle control group by Dunn's or Shirley's test
[a] Data are presented as µg/mL (mean ± standard error). No data were available for the 125 mg/kg males or females due to 100% mortality.
[b] Below the limit of detection (1.30 µg/mL)
[c] n=10

TABLE F5
Urine 3-Hydroxypropyl Mercapturic Acid Concentrations for Mice in the 14-Week Gavage Study of Allyl Alcohol[a]

	Vehicle Control	3 mg/kg	6 mg/kg	12 mg/kg	25 mg/kg	50 mg/kg
n	2	2	2	2	2	2
Male						
1st dose	7.72 ± 0.35	63.80 ± 12.70	58.35 ± 11.35	113.85 ± 54.15*	253.00 ± 7.00**	407.00 ± 174.00*
45th dose	15.05 ± 1.55	41.35 ± 5.95	63.70 ± 13.00*	118.50 ± 12.50*	161.50 ± 31.50*	328.50 ± 17.50**
Female						
1st dose	—[b,c]	22.50 ± 0.70**	35.75 ± 4.25**	48.95 ± 0.65**	158.00 ± 32.00**	229.00 ± 47.00**
45th dose	—[c]	17.50 ± 1.20**	35.60 ± 3.10**	69.75 ± 8.25**	118.00 ± 1.00**	252.50 ± 1.50**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test
** $P \leq 0.01$
[a] Data are presented as µg/mL (mean ± standard error).
[b] Below the limit of detection (1.30 µg/mL)
[c] n=10

TABLE F6
Urine 3-Hydroxypropyl Mercapturic Acid Concentrations for Mice in the 14-Week Gavage Study of Acrolein[a]

	Vehicle Control	1.25 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
n	2	2	2	2	2
Male					
1st dose	12.15 ± 1.65	28.00 ± 5.70	39.00 ± 5.70	65.00 ± 10.90*	68.20 ± 3.10*
45th dose	13.45 ± 0.85	25.10 ± 7.90	36.90 ± 2.40*	62.20 ± 5.50*	100.55 ± 7.45**
Female					
1st dose	—[b,c]	22.70 ± 3.70**	32.10 ± 0.80**	34.35 ± 16.05**	35.55 ± 7.95**
45th dose	—[c]	12.30 ± 1.00**	33.85 ± 0.85**	42.45 ± 4.65**	73.15 ± 11.85**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test
** $P \leq 0.01$
[a] Data are presented as µg/mL (mean ± standard error). No data were available for the 20 mg/kg males or females due to 100% mortality.
[b] Below the limit of detection (1.30 µg/mL)
[c] n=10

APPENDIX G
GENETIC TOXICOLOGY

TABLE G1
Mutagenicity of Allyl Acetate in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b]				
		-S9			+30% hamster S9	+30% rat S9
		Trial 1	Trial 2	Trial 3		
Study performed at SRI International						
TA100	0	115 ± 3.2	92 ± 7.5	80 ± 1.5	114 ± 3.5	124 ± 3.5
	3				101 ± 10.9	120 ± 2.6
	10				104 ± 5.1	113 ± 1.5
	33	118 ± 2.3			111 ± 5.0	115 ± 3.5
	100	108 ± 7.1			99 ± 11.9	120 ± 3.5
	333	111 ± 2.2			104 ± 9.4	87 ± 7.3
	666		89 ± 12.8	83 ± 3.0		
	1,000	112 ± 2.9	89 ± 8.6	78 ± 2.3		
	1,666		105 ± 2.0	95 ± 2.5		
	3,333	157 ± 10.9	143 ± 9.9	116 ± 4.6		
	6,666		172 ± 10.9	141 ± 5.5		
Trial summary		Equivocal	Weakly Positive	Weakly Positive	Negative	Negative
Positive control[c]		870 ± 14.7	900 ± 50.9	829 ± 11.4	415 ± 10.5	366 ± 7.6
TA1535	0	12 ± 1.5	9 ± 1.5		12 ± 2.2	14 ± 3.2
	3				11 ± 1.8	12 ± 1.5
	10				9 ± 0.6	13 ± 2.2
	33	9 ± 2.2			10 ± 0.9	10 ± 1.8
	100	10 ± 3.3			10 ± 0.7	10 ± 2.0
	333	16 ± 0.9	15 ± 0.9		11 ± 1.5	10 ± 1.5
	666		21 ± 4.2			
	1,000	27 ± 3.2	33 ± 4.4			
	1,666		38 ± 5.2			
	3,333	42 ± 2.3	63 ± 3.5			
Trial summary		Positive	Positive		Negative	Negative
Positive control		851 ± 15.6	732 ± 7.9		198 ± 14.3	138 ± 8.4
TA97	0	151 ± 46.9			145 ± 9.1	137 ± 4.3
	3				143 ± 6.9	121 ± 3.3
	10				140 ± 3.8	131 ± 5.2
	33	120 ± 11.4			134 ± 2.9	130 ± 2.3
	100	123 ± 6.7			123 ± 12.7	129 ± 4.6
	333	103 ± 8.3			106 ± 7.7	107 ± 2.3
	1,000	112 ± 4.9				
	3,333	127 ± 13.7				
Trial summary		Negative			Negative	Negative
Positive control		392 ± 11.9			409 ± 8.5	374 ± 10.7
TA98	0	15 ± 1.2			20 ± 1.5	25 ± 2.3
	3				25 ± 3.8	16 ± 2.1
	10				21 ± 1.5	18 ± 1.8
	33	14 ± 0.9			26 ± 2.9	22 ± 1.9
	100	15 ± 3.4			21 ± 2.6	19 ± 1.2
	333	18 ± 1.9			16 ± 1.8	17 ± 1.5
	1,000	14 ± 2.2				
	3,333	17 ± 2.0				
Trial summary		Negative			Negative	Negative
Positive control		374 ± 13.0			328 ± 21.0	187 ± 15.1

TABLE G1
Mutagenicity of Allyl Acetate in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate -S9 Trial 1	-S9 Trial 2	+30% hamster S9	+30% rat S9
Study performed at Environmental Health Research and Testing, Inc.					
TA100	0	100 ± 2.6	97 ± 2.3	113 ± 6.6	117 ± 3.5
	10			108 ± 3.2	117 ± 2.0
	33			109 ± 3.1	113 ± 2.9
	100			114 ± 3.1	107 ± 3.5
	333	98 ± 1.5	100 ± 2.1	68 ± 3.0[d]	64 ± 2.6[d]
	1,000	104 ± 4.1	104 ± 2.3	Toxic	Toxic
	3,333	131 ± 3.7	129 ± 4.1		
	6,666	178 ± 2.3	180 ± 7.8		
	10,000	187 ± 4.4	175 ± 5.0		
Trial summary		Weakly Positive	Weakly Positive	Negative	Negative
Positive control		635 ± 5.0	591 ± 11.4	666 ± 11.1	578 ± 5.5
TA1535	0	16 ± 2.6			
	333	20 ± 2.3			
	1,000	27 ± 0.9			
	3,333	57 ± 2.4			
	6,666	128 ± 5.7			
	10,000	139 ± 2.5			
Trial summary		Positive			
Positive control		307 ± 4.1			
TA98	0	19 ± 2.6		27 ± 3.7	28 ± 1.5
	10			28 ± 1.7	26 ± 1.5
	33			30 ± 1.5	23 ± 2.9
	100			29 ± 2.0	23 ± 1.2
	333	18 ± 1.3		15 ± 1.8	10 ± 1.7[d]
	1,000	21 ± 0.7		Toxic	Toxic
	3,333	22 ± 1.7			
	6,666	17 ± 2.5			
	10,000	17 ± 1.2			
Trial summary		Negative		Negative	Negative
Positive control		317 ± 9.2		446 ± 21.7	349 ± 17.4

[a] The detailed protocol is presented by Mortelmans *et al.* (1986). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[d] Slight toxicity

TABLE G2
Mutagenicity of Allyl Alcohol in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b]					
		−S9		+hamster S9		+rat S9	
		Trial 1	Trial 2	10%	30%	10%	30%
Study performed at Environmental Health Research and Testing, Inc.							
TA100	0	108 ± 2.4	125 ± 2.6	140 ± 2.6	139 ± 2.0	141 ± 2.6	108 ± 3.2
	3	110 ± 4.4	127 ± 3.3	138 ± 3.2	135 ± 3.2	138 ± 2.9	109 ± 2.7
	10	119 ± 1.2	126 ± 3.6	136 ± 3.2	130 ± 6.2	138 ± 3.8	111 ± 3.4
	33	112 ± 0.7	128 ± 3.5	141 ± 1.5	133 ± 2.6	143 ± 2.6	109 ± 1.3
	100	79 ± 2.7[c]	116 ± 2.3	135 ± 3.0	121 ± 2.7	124 ± 2.9	83 ± 6.5
	333	Toxic	107 ± 3.1[c]	94 ± 4.1[c]	70 ± 3.8[c]	115 ± 3.5[c]	78 ± 5.6[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		891 ± 5.7	890 ± 12.5	1,009 ± 7.5	766 ± 17.0	961 ± 36.1	783 ± 9.3
TA1535	0	15 ± 1.2	14 ± 1.2	12 ± 1.5	17 ± 2.4	10 ± 1.8	16 ± 2.0
	3	14 ± 1.2	15 ± 0.9	13 ± 0.3	19 ± 1.2	9 ± 1.5	14 ± 2.0
	10	16 ± 1.7	13 ± 1.5	11 ± 2.1	16 ± 2.0	12 ± 2.1	17 ± 1.3
	33	15 ± 2.3	13 ± 0.3	11 ± 1.5	15 ± 0.7	10 ± 2.0	14 ± 1.7
	100	14 ± 2.1	11 ± 2.1	11 ± 2.9	14 ± 1.5	11 ± 1.2	15 ± 2.0
	333	Toxic	Toxic	5 ± 1.2[c]	5 ± 1.8[c]	6 ± 2.3[c]	7 ± 1.2[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		335 ± 3.8	535 ± 7.2	367 ± 4.3	372 ± 4.6	346 ± 6.8	351 ± 4.8
TA97	0	98 ± 2.0	93 ± 2.5	99 ± 1.8	112 ± 2.3	125 ± 2.2	120 ± 4.0
	3	103 ± 3.8	94 ± 2.0	106 ± 2.6	112 ± 2.5	116 ± 3.6	121 ± 2.6
	10	103 ± 3.5	95 ± 1.8	108 ± 3.8	111 ± 2.0	96 ± 3.2	116 ± 3.2
	33	102 ± 2.6	98 ± 1.8	102 ± 3.5	102 ± 5.0	127 ± 3.5	118 ± 4.7
	100	95 ± 2.7	92 ± 2.3	99 ± 3.2	108 ± 2.0	125 ± 2.6	120 ± 2.6
	333	Toxic	Toxic	84 ± 4.4[c]	108 ± 3.2	95 ± 3.5[c]	77 ± 2.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		508 ± 11.9	625 ± 26.1	938 ± 5.5	572 ± 20.4	537 ± 6.4	604 ± 15.3
TA98	0	31 ± 2.6	18 ± 0.9	46 ± 1.5	41 ± 2.1	43 ± 2.4	38 ± 1.7
	3	31 ± 2.3	18 ± 1.3	46 ± 3.5	40 ± 2.6	40 ± 4.4	41 ± 0.6
	10	27 ± 1.9	17 ± 2.3	47 ± 2.4	42 ± 2.6	45 ± 2.3	35 ± 1.2
	33	29 ± 0.3	20 ± 3.2	45 ± 3.3	39 ± 2.3	44 ± 1.9	32 ± 3.5
	100	17 ± 1.5[c]	16 ± 1.7	46 ± 3.8	25 ± 2.6	37 ± 2.0	26 ± 2.6
	333	Toxic	4 ± 1.2[c]	16 ± 1.8[c]	12 ± 2.3[c]	16 ± 3.1[c]	Toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		252 ± 6.8	429 ± 5.5	980 ± 3.8	954 ± 15.2	931 ± 3.5	577 ± 6.1

TABLE G2
Mutagenicity of Allyl Alcohol 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							
TA100	0	169 ± 6.7	103 ± 2.4	96 ± 5.9	177 ± 7.7	113 ± 1.5	181 ± 1.2
	0.3	175 ± 8.5					
	1	183 ± 2.7	100 ± 2.4		183 ± 10.0		188 ± 5.9
	3	166 ± 2.0	101 ± 4.5	111 ± 11.7	179 ± 3.2	101 ± 7.3	181 ± 8.1
	10	163 ± 9.5	105 ± 3.3	102 ± 6.7	198 ± 6.7	107 ± 0.3	184 ± 5.5
	33	144 ± 4.4	97 ± 5.9	105 ± 5.1	160 ± 14.4	111 ± 2.1	175 ± 5.9
	66		73 ± 3.1				
	100			108 ± 8.1	155 ± 4.3	100 ± 5.8	153 ± 2.3
	166			48 ± 8.2		49 ± 9.8	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		943 ± 20.4	647 ± 32.4	619 ± 1.8	954 ± 6.5	484 ± 22.4	1,096 ± 52.9
TA1535	0	8 ± 2.3	15 ± 1.5	12 ± 2.5	10 ± 1.2	17 ± 0.6	11 ± 1.7
	0.3	9 ± 0.3					
	1	8 ± 1.9	14 ± 2.3		10 ± 2.4		14 ± 2.3
	3	9 ± 1.9	15 ± 1.5	11 ± 1.7	12 ± 2.6	13 ± 2.3	12 ± 2.2
	10	9 ± 1.3	13 ± 2.2	10 ± 2.3	8 ± 0.9	8 ± 0.0	8 ± 0.6
	33	10 ± 2.8	10 ± 1.2	12 ± 2.3	8 ± 0.6	8 ± 0.7	9 ± 1.5
	66		10 ± 2.7				
	100			11 ± 2.1	11 ± 1.0	8 ± 2.3	7 ± 0.6
	166			8 ± 0.3		5 ± 0.7	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		629 ± 17.0	812 ± 29.4	125 ± 24.7	468 ± 7.6	123 ± 6.5	230 ± 5.6
TA97	0	115 ± 9.7	170 ± 4.0	179 ± 9.2	170 ± 2.6	174 ± 9.8	175 ± 12.2
	0.3	129 ± 8.4					
	1	124 ± 4.8	171 ± 6.7		166 ± 9.1		171 ± 6.7
	3	131 ± 8.3	178 ± 12.5	188 ± 2.1	165 ± 6.0	183 ± 9.5	179 ± 14.5
	10	123 ± 3.8	170 ± 8.0	187 ± 4.1	163 ± 4.6	192 ± 4.7	187 ± 2.1
	33	117 ± 13.7	150 ± 13.0	189 ± 5.2	142 ± 2.3	185 ± 3.5	169 ± 5.8
	66		106 ± 5.5				
	100			167 ± 9.3	155 ±10.5	159 ± 3.2	151 ± 20.2
	166			105 ± 12.7		83 ± 9.2	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		426 ± 35.3	515 ± 13.2	545 ± 22.3	657 ± 33.0	478 ± 12.3	518 ± 30.6

TABLE G2
Mutagenicity of Allyl Alcohol in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate					
		−S9		+hamster S9		+rat S9	
		Trial 1	Trial 2	10%	30%	10%	30%
Study performed at SRI International (continued)							
TA98	0	19 ± 2.3	20 ± 6.0	20 ± 3.2	23 ± 3.3	18 ± 1.7	25 ± 3.2
	0.3	15 ± 0.9					
	1	18 ± 0.9	16 ± 0.9		22 ± 2.5		19 ± 4.5
	3	16 ± 3.5	17 ± 3.0	22 ± 3.2	16 ± 0.9	23 ± 2.9	18 ± 3.8
	10	15 ± 0.7	17 ± 0.7	17 ± 1.7	18 ± 2.4	18 ± 3.5	14 ± 0.9
	33	12 ± 1.7	15 ± 1.2	14 ± 2.4	19 ± 1.0	16 ± 1.0	17 ± 0.7
	66		12 ± 1.2				
	100			10 ± 0.7	17 ± 1.5	10 ± 1.0	10 ± 0.9
	166			9 ± 0.6		12 ± 2.3	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		373 ± 6.7	306 ± 5.5	452 ± 11.4	362 ± 28.2	340 ± 17.2	641 ± 25.0

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

TABLE G3
Mutagenicity of Acrolein in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b]				
		−S9	+10% hamster S9		+10% rat S9	
			Trial 1	Trial 2	Trial 1	Trial 2
Study performed at EG&G Mason Research Institute						
TA100	0	125 ± 10.3	129 ± 6.1	105 ± 11.8	137 ± 7.0	131 ± 8.4
	0.03	142 ± 3.0				
	0.1	136 ± 2.3				
	0.3	131 ± 4.6				
	1	137 ± 5.5		109 ± 8.4		117 ± 9.5
	3.3	129 ± 5.2		116 ± 5.2		127 ± 3.8
	10		136 ± 4.4	107 ± 6.7	150 ± 3.2	114 ± 9.7
	15		137 ± 13.4		138 ± 4.6	
	20		149 ± 7.0		133 ± 8.2	
	25		133 ± 2.2[c]		136 ± 3.2[c]	
	33		138 ± 6.4[c]	181 ± 21.5[c]	171 ± 8.3[c]	268 ± 20.0[c]
	40		148 ± 7.4[c]		197 ± 7.0[c]	
	50		173 ± 1.3[c]		245 ± 4.8[c]	
	100		42 ± 5.9[c]	Toxic	44 ± 5.6[c]	Toxic
Trial summary		Negative	Equivocal	Equivocal	Positive	Equivocal
Positive control[d]		1,443 ± 17.0	2,061 ± 106.0	1,577 ± 15.1	1,154 ± 42.9	1,426 ± 44.6
TA1535	0	27 ± 3.2	11 ± 0.7		14 ± 2.3	
	0.1	27 ± 2.6				
	0.3	20 ± 2.3				
	1	24 ± 2.1	8 ± 0.9		11 ± 2.3	
	3.3	19 ± 3.7[c]	10 ± 0.7		7 ± 0.3	
	10	Toxic	10 ± 2.7		12 ± 2.3	
	33		19 ± 0.3		15 ± 0.0[c]	
	100		Toxic		Toxic	
Trial summary		Negative	Equivocal		Negative	
Positive control		1,417 ± 12.9	180 ± 5.2		51 ± 6.1	
TA1537	0	7 ± 2.3	9 ± 2.1		7 ± 1.3	
	0.1	7 ± 0.6				
	0.3	6 ± 1.9				
	1	12 ± 1.2	8 ± 1.5		6 ± 2.5	
	3.3	5 ± 1.0[c]	6 ± 2.4		6 ± 1.7	
	10	Toxic	9 ± 3.1		11 ± 1.0	
	33		5 ± 1.2		10 ± 1.2[c]	
	100		Toxic		Toxic	
Trial summary		Negative	Negative		Negative	
Positive control		410 ± 99.4	141 ± 2.6		115 ± 13.0	
TA98	0	22 ± 3.8	29 ± 2.2		25 ± 1.8	
	0.1	24 ± 3.2				
	0.3	23 ± 1.5				
	1	20 ± 2.9	29 ± 4.0		24 ± 3.3	
	3.3	Toxic	29 ± 0.3		24 ± 4.3	
	10	Toxic	22 ± 1.0		21 ± 1.8[c]	
	33		25 ± 4.2[c]		30 ± 3.4[c]	
	100		10 ± 0.5[c]		Toxic	
Trial summary		Negative	Negative		Negative	
Positive control		1,240 ± 37.1	1,373 ± 34.9		1,260 ± 13.2	

TABLE G3
Mutagenicity of Acrolein in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate −S9 Trial 1	−S9 Trial 2	+hamster S9 5%	+hamster S9 10%	+hamster S9 30%	+hamster S9 30%
Study performed at SRI International							
TA100	0	127 ± 17.1	85 ± 5.3	105 ± 4.8	121 ± 8.70	115 ± 7.6	133 ± 2.5
	0.01		90 ± 5.2				
	0.03		95 ± 11.0				
	0.1	141 ± 19.7	98 ± 4.4				
	0.3	148 ± 4.0	84 ± 1.9				
	1	155 ± 8.5	80 ± 4.2	114 ± 16.5	120 ± 3.2	127 ± 14.7	134 ± 4.4
	3	143 ± 29.8		105 ± 8.5	110 ± 1.5	130 ± 7.5	121 ± 2.7
	6	117 ± 39.1[c]		114 ± 6.6	95 ± 8.4	130 ± 3.2	121 ± 5.7
	10			97 ± 3.5	106 ± 12.6	125 ± 6.1	105 ± 6.6
	16			99 ± 1.5	94 ± 7.1	129 ± 6.4	126 ± 7.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		906 ± 6.0	390 ± 21.9	671 ± 78.1	799 ± 40.3	595 ± 47.8	491 ± 55.5

Strain	Dose (µg/plate)	+rat S9 5%	+rat S9 10%	+rat S9 30%	+rat S9 30%
TA100	0	104 ± 8.5	114 ± 7.8	143 ± 7.3	143 ± 14.5
	1	98 ± 11.0	130 ± 4.3	167 ± 7.9	134 ± 5.1
	3	113 ± 12.2	120 ± 7.4	150 ± 2.5	120 ± 5.8
	6	112 ± 5.1	116 ± 9.2	159 ± 10.0	147 ± 4.1
	10	106 ± 5.3	101 ± 2.3	150 ± 4.8	133 ± 3.2
	16	108 ± 10.3	110 ± 14.3	157 ± 13.0	131 ± 4.7
Trial summary		Negative	Negative	Negative	Negative
Positive control		636 ± 66.8	361 ± 21.5	405 ± 1.5	398 ± 3.2

Strain	Dose (µg/plate)	+30% hamster S9	+30% rat S9
TA1535	0	12 ± 0.9	16 ± 3.4
	1	7 ± 1.5	12 ± 0.7
	3	6 ± 2.0	10 ± 1.5
	6	8 ± 0.3	12 ± 1.5
	10	9 ± 1.3	8 ± 1.9
	16	6 ± 1.5	10 ± 1.3
Trial summary		Negative	Negative
Positive control		209 ± 6.4	79 ± 2.7

TABLE G3
Mutagenicity of Acrolein in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate +30% hamster S9	Revertants/Plate +30% rat S9
Study performed at SRI International (continued)			
TA1538	0	12 ± 3.8	12 ± 2.4
	1	13 ± 3.4	11 ± 1.2
	3	10 ± 1.5	13 ± 1.9
	6	10 ± 1.8	11 ± 1.8
	10	13 ± 3.1	11 ± 1.5
	16	12 ± 3.8	9 ± 0.7
Trial summary		Negative	Negative
Positive control		518 ± 61.1	122 ± 13.0

		−S9		+hamster S9			
		Trial 1	**Trial 2**	**5%**	**10%**	**30%**	**30%**
TA98	0	16 ± 3.8	18 ± 1.3	32 ± 3.8	20 ± 4.0	24 ± 4.4	35 ± 2.9
	0.01	26 ± 1.2	14 ± 3.5				
	0.03	34 ± 3.4	16 ± 2.9				
	0.1	26 ± 1.2	24 ± 3.3				
	0.3	23 ± 1.0	18 ± 3.9				
	1	33 ± 4.4	18 ± 1.8	24 ± 2.3	21 ± 1.5	16 ± 1.8	24 ± 2.6
	3			27 ± 0.6	23 ± 2.2	18 ± 1.2	22 ± 3.4
	6			28 ± 2.3	22 ± 3.5	23 ± 1.7	22 ± 3.8
	10			29 ± 1.2	23 ± 2.7	19 ± 1.0	29 ± 5.9
	16			26 ± 2.9	26 ± 1.9	24 ± 7.8	34 ± 1.2
Trial summary		Equivocal	Negative	Negative	Negative	Negative	Negative
Positive control		575 ± 17.3	472 ± 41.9	731 ± 41.5	603 ± 18.8	502 ± 19.0	234 ± 24.2

		+rat S9			
		5%	**10%**	**30%**	**30%**
TA98	0	30 ± 1.5	26 ± 1.9	23 ± 2.2	29 ± 6.1
	1	24 ± 4.4	24 ± 0.6	14 ± 1.2	23 ± 3.8
	3	24 ± 2.0	27 ± 2.1	19 ± 5.9	22 ± 3.1
	6	22 ± 3.4	23 ± 1.8	14 ± 2.7	18 ± 4.4
	10	27 ± 2.6	26 ± 1.3	12 ± 1.9	23 ± 3.4
	16	26 ± 1.2	22 ± 3.5	15 ± 2.1	20 ± 2.6
Trial summary		Negative	Negative	Negative	Negative
Positive control		537 ± 90.3	277 ± 29.2	152 ± 12.3	107 ± 3.8

TABLE G3
Mutagenicity of Acrolein in *Salmonella typhimurium*

Strain	Dose (mL/chamber)	Revertants/Plate −S9 Trial 1	Revertants/Plate −S9 Trial 2
Study performed at SRI International using a desiccator protocol			
TA100	0	147 ± 12.2	144 ± 4.8
	0.025	120 ± 5.2	123 ± 9.4
	0.05	109 ± 7.8	132 ± 10.8
	0.1	140 ± 15.6	124 ± 5.8
	0.25	125 ± 24.6	80 ± 4.3
	0.5	0 ± 0.0[c]	
Trial summary		Negative	Negative
Positive control		576 ± 49.4	661 ± 4.3

Strain	Dose (mL/chamber)	+hamster S9 5%	+hamster S9 10%	+hamster S9 10%	+hamster S9 30%	+hamster S9 30%	+hamster S9 30%
TA100	0	158 ± 2.3	136 ± 1.2	133 ± 4.4	117 ± 8.1	135 ± 19.5	115 ± 1.0
	0.005	114 ± 11.3	140 ± 17.3			168 ± 13.9	109 ± 11.0
	0.01	115 ± 8.0	150 ± 6.7			160 ± 6.9	110 ± 4.4
	0.025	117 ± 17.4	137 ± 7.6			143 ± 3.3	121 ± 6.4
	0.05	86 ± 5.7	142 ± 9.7	132 ± 4.6	155 ± 9.0	147 ± 8.0	122 ± 6.2
	0.1	122 ± 10.9	128 ± 8.5	129 ± 3.5	160 ± 7.4	138 ± 8.0	125 ± 1.2
	0.25	102 ± 6.1	115 ± 7.0	123 ± 11.1	142 ± 7.7	128 ± 6.8	
	0.5			112 ± 2.0	147 ± 3.2		
	1				104 ± 2.9		
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Negative
Positive control		803 ± 42.3	704 ± 18.0	1,058 ± 43.6	690 ± 18.7	492 ± 18.5	437 ± 41.9

Strain	Dose (mL/chamber)	+rat S9 5%	+rat S9 10%	+rat S9 10%	+rat S9 30%	+rat S9 30%	+rat S9 30%
TA100	0	148 ± 6.2	149 ± 11.2	142 ± 8.3	161 ± 9.8	169 ± 5.5	105 ± 1.7
	0.005	132 ± 4.7	135 ± 2.6			173 ± 11.9	137 ± 11.7
	0.01	122 ± 5.5	161 ± 11.3			161 ± 5.9	124 ± 0.3
	0.025	139 ± 7.7	131 ± 10.5			156 ± 3.2	135 ± 2.0
	0.05	124 ± 3.8	99 ± 3.3	130 ± 20.0	198 ± 11.8	164 ± 7.9	136 ± 3.7
	0.1	137 ± 4.5	111 ± 11.0	126 ± 7.4	198 ± 2.0	174 ± 4.4	139 ± 0.3
	0.25	126 ± 8.7	133 ± 2.5	127 ± 0.9	181 ± 11.5	170 ± 8.8	
	0.5			50 ± 12.2	158 ± 7.8		
	1				83 ± 16.9[c]		
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Negative
Positive control		552 ± 7.6	368 ± 8.2	1,064 ± 83.8	315 ± 55.5	369 ± 16.7	408 ± 13.7

TABLE G3
Mutagenicity of Acrolein in *Salmonella typhimurium*

Strain	Dose (mL/chamber)	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 using a desiccator protocol (continued)							
TA1535	0	8 ± 1.2	7 ± 1.5	13 ± 1.5	13 ± 1.8	12 ± 0.6	16 ± 4.4
	0.025	11 ± 0.3	8 ± 0.3				
	0.05	14 ± 1.7	10 ± 2.0	9 ± 1.7	11 ± 0.9	8 ± 1.2	14 ± 3.5
	0.1	10 ± 1.2	7 ± 1.7	10 ± 0.9	11 ± 0.6	15 ± 2.7	12 ± 1.3
	0.25	8 ± 2.2	1 ± 0.6[c]	12 ± 1.2	19 ± 2.4	9 ± 1.8	13 ± 1.5
	0.5			10 ± 1.2	19 ± 1.7	4 ± 1.2	13 ± 0.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		783 ± 15.0	532 ± 26.4	111 ± 3.2	148 ± 7.6	94 ± 4.2	102 ± 10.5
TA97	0	227 ± 2.2	224 ± 11.1	196 ± 13.2	216 ± 4.9	206 ± 7.4	238 ± 3.9
	0.025	215 ± 2.0	172 ± 4.7				
	0.05	203 ± 12.0	165 ± 4.6	213 ± 3.8	227 ± 16.9	189 ± 10.1	218 ± 7.6
	0.1	203 ± 5.5	165 ± 7.9	205 ± 7.5	224 ± 2.1	225 ± 1.5	261 ± 10.9
	0.25	214 ± 4.2	11 ± 4.7	203 ± 8.0	221 ± 1.5	217 ± 9.7	259 ± 7.1
	0.5			137 ± 13.3	195 ± 15.9	39 ± 7.7	242 ± 4.7
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		459 ± 23.7	480 ± 16.0	639 ± 31.8	430 ± 16.8	376 ± 5.5	374 ± 15.8
TA98	0	26 ± 5.0	24 ± 1.7	25 ± 2.9	28 ± 1.7	23 ± 5.6	29 ± 0.7
	0.025	28 ± 1.2	26 ± 5.0				
	0.05	25 ± 2.6	26 ± 0.6	17 ± 3.0	19 ± 1.2	17 ± 0.3	26 ± 6.2
	0.1	24 ± 2.3	28 ± 0.3	21 ± 0.9	20 ± 2.1	23 ± 2.1	20 ± 3.8
	0.25	16 ± 2.7[c]	32 ± 2.9	17 ± 1.8	19 ± 5.0	16 ± 2.8	28 ± 5.9
	0.5	3 ± 1.5[c]		15 ± 3.5	20 ± 2.2	8 ± 3.5	23 ± 1.3
	1				14 ± 3.3		5 ± 0.3[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		570 ± 27.5	945 ± 8.6	725 ± 44.3	358 ± 18.1	283 ± 5.5	90 ± 9.8

[a] The detailed protocol and the EG&G Mason Research Institute data are presented by Haworth *et al.* (1983); the protocols for the SRI International studies are described by Zeiger *et al.* (1992). 0 µg/plate was the solvent control (preincubation protocol); 0 mL/chamber was the vapor control (desiccator protocol).
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97 and TA1537), and 4-nitro-*o*-phenylenediamine (TA98 and TA1538). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE G4
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Acrolein[a]

Compound	Concentration (μg/mL)	Total Cells Scored	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Summary: Weakly positive								
Distilled water[c]		50	1,048	404	0.38	8.1	26.0	
Acrolein	0.1	50	1,048	464	0.44	9.3	26.0	14.85
	0.3	50	1,045	482	0.46	9.6	26.0	19.65
	1	50	1,047	519	0.49	10.4	26.0	28.59*
					P<0.000[d]			
Triethylenemelamine[e]	0.015	50	1,048	1,494	1.42	29.9	26.0	269.81
+S9								
Summary: Negative								
Distilled water		50	1,042	430	0.41	8.6	26.0	
Acrolein	0.1	50	1,050	511	0.48	10.2	26.0	17.93
	0.3	50	1,045	458	0.43	9.2	26.0	6.21
	1	50	1,028	476	0.46	9.5	26.0	12.21
					P=0.132			
Cyclophosphamide[e]	1	50	1,039	1,125	1.08	22.5	26.0	162.39

* Positive response (≥20% increase over solvent control)
[a] Study was performed at Columbia University. The detailed protocol and these data are presented by Galloway *et al.* (1987). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine.
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in vehicle control cells
[c] Vehicle control
[d] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose
[e] Positive control

TABLE G5
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Acrolein[a]

Compound	Concentration (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
-S9					
Harvest time: 14 hours					
Summary: Negative					
Distilled water[b]		100	1	0.01	1.0
Acrolein	0.1	100	2	0.02	2.0
	0.3	100	2	0.02	2.0
	1	100	5	0.05	5.0
					P=0.042[c]
Triethylenemelamine[d]	0.15	100	32	0.32	27.0
+S9					
Harvest time: 14 hours					
Summary: Negative					
Distilled water		100	0	0.00	0.0
Acrolein	0.1	100	2	0.02	2.0
	0.3	100	3	0.03	2.0
	1	100	5	0.05	3.0
					P=0.062
Cyclophosphamide[d]	15	100	47	0.47	33.0

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

TABLE G6
Induction of Sex-Linked Recessive Lethal Mutations on *Drosophila melanogaster* by Acrolein[a]

Route of Exposure	Dose (ppm)	Incidence of Death (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested			
				Mating 1	Mating 2	Mating 3	Total[b]
Larva feeding							
	802	34	0	3/2,991	1/2,439		4/5,430 (0.07%)
	0			1/1,789	2/2,208		3/3,997 (0.08%)
Adult treatments							
Feeding	3,000	10	0	3/2,644	2/2,337	0/2,019	5/7,040 (0.07%)
	0			2/2,103	1/1,945	0/1,850	3/5,898 (0.05%)
Injection	200	19	1	3/2,032	0/1,947	0/1,842	3/5,821 (0.05%)
	0			1/2,025	2/1,960	1/1,857	4/5,842 (0.07%)

[a] Study was performed at the University of Wisconsin, Madison. The detailed protocol and data for adult experiments are presented by Zimmering *et al.* (1985). The protocol and data for the larval experiment are presented by Zimmering *et al.* (1989). Results were not significant at the $P \leq 0.05$ level (Margolin *et al.*, 1983). The mean mutant frequency from 518 negative control experiments is 0.074% (Mason *et al.*, 1992).

[b] Total number of lethal mutations/total number of X chromosomes tested for three mating trials

TABLE G7
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with Allyl Acetate by Gavage[a]

Compound	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]
Corn oil[c]		5	1.2 ± 0.4
Allyl Acetate	9.38	5	0.7 ± 0.2
	18.75	5	1.5 ± 0.4
	37.5	5	1.4 ± 0.3
	75	5	1.7 ± 0.3
	150	3	1.3 ± 0.2
	300	Lethal	—
			P=0.189[d]
Cyclophosphamide[e]	10	5	10.0 ± 0.9

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Shelby *et al.* (1993).
PCE=polychromatic erythrocyte.
[b] Mean ± standard error
[c] Vehicle control
[d] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)
[e] Positive control

TABLE G8
Induction of Micronuclei on Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with Allyl Alcohol by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]
Phosphate-buffered saline[c]		4	1.4 ± 0.2
Allyl Alcohol	5	5	2.0 ± 0.2
	10	5	1.6 ± 0.3
	20	5	1.4 ± 0.2
	40	Lethal	—
			P=0.649[d]
Cyclophosphamide[e]	7.5	5	24.2 ± 0.8

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Shelby *et al.* (1993).
PCE=polychromatic erythrocyte.
[b] Mean ± standard error
[c] Vehicle control
[d] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)
[e] Positive control

TABLE G9
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Allyl Acetate by Gavage for 14 Weeks[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs (%)
Male					
Methylcellulose (0.5%)[d]		9	1.22 ± 0.28		2.7
Allyl Acetate					
	8	7	2.14 ± 0.46	0.0758	2.5
	16	10	2.20 ± 0.33	0.0530	2.7
	32	9	2.33 ± 0.41	0.0384	2.6
	62.5	2	3.50 ± 0.50[e]	0.0113	2.5
			P=0.064[f]		
Female					
Methylcellulose (0.5%)		9	0.89 ± 0.26		2.7
Allyl Acetate					
	8	8	1.50 ± 0.42	0.1230	2.4
	16	6	1.17 ± 0.40	0.2990	2.4
	32	7	1.00 ± 0.22	0.4099	2.3
	62.5	6	3.17 ± 0.40	0.0006	2.4
			P=0.001		

[a] Study performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte. PCE=polychromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control; significant at P≤0.006 (ILS, 1990))
[d] Vehicle control
[e] Excluded from trend test because fewer than three animals survived
[f] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE G10
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Allyl Alcohol by Gavage for 14 Weeks[a]

Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs (%)
Male				
Methylcellulose (0.5%)[d]	10	1.10 ± 0.38		2.3
Allyl Alcohol				
3	10	1.20 ± 0.25	0.4174	2.6
6	10	1.70 ± 0.40	0.1283	2.2
12	10	1.40 ± 0.34	0.2741	2.2
25	10	1.20 ± 0.33	0.4174	2.4
50	10	1.60 ± 0.45	0.1678	2.2
		P=0.273[e]		
Female				
Methylcellulose (0.5%)	10	0.70 ± 0.21		2.3
Allyl Alcohol				
3	10	0.90 ± 0.28	0.3085	2.5
6	10	1.00 ± 0.21	0.2333	2.2
12	10	0.70 ± 0.26	0.5000	2.2
25	10	1.50 ± 0.31	0.0440	2.1
50	9	1.11 ± 0.26	0.1720	2.2
		P=0.118		

[a] Study performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte. PCE=polychromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the 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)

TABLE G11
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Acrolein by Gavage for 14 Weeks[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]
Male				
Methylcellulose (0.5%)[d]		10	1.00 ± 0.11	
Acrolein	1.25	9	1.06 ± 0.23	0.4329
	2.50	10	0.75 ± 0.20	0.8011
	5.00	9	1.11 ± 0.18	0.3694
	10.00	9	1.28 ± 0.12	0.2106
			P=0.137[e]	
Female				
Methylcellulose (0.5%)		9	0.50 ± 0.12	
Acrolein	1.25	10	0.60 ± 0.15	0.3394
	2.50	10	0.50 ± 0.13	0.5000
	5.00	9	1.00 ± 0.24	0.0416
	10.00	7	0.57 ± 0.13	0.3916
			P=0.249	

[a] Study performed at SITEK Research Laboratories. 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≤0.006 (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 H
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Allyl acetate (lot 04525EF), allyl alcohol (lot 00501TF), and acrolein (lot 11163AG) were obtained from Aldrich Chemical Company (Milwaukee, WI). Information on identity and purity were provided by the manufacturer; identity of all chemicals and purity of acrolein were confirmed by the study laboratory, Battelle Columbus Laboratories (Columbus, OH). Reports on analyses performed in support of the allyl acetate, allyl alcohol, and acrolein studies are on file at the National Institute of Environmental Health Sciences.

Allyl acetate and allyl alcohol, colorless liquids, and acrolein, a yellow liquid, were identified by infrared spectroscopy. Each spectrum was consistent with a literature reference (*Aldrich*, 1985) and with that expected for the structure.

Gas chromatography data provided by the manufacturer indicated a purity of 99.3% for allyl acetate, 98.8% for allyl alcohol, and 98.8% for acrolein. Titration data from the manufacturer indicated 7.74% water for acrolein.

The purity of acrolein was determined by the study laboratory using gas chromatography with a flame ionization detector and helium as the carrier gas at a flow rate of 10 mL/minute. The system used a 1% SP 1000 60/80 Carbopack B column with an isothermal oven temperature of 150° C. Gas chromatographic analyses indicated no organic impurities at significant concentrations. The combined data from the manufacturer and study laboratory indicated an overall purity of greater than 90% for acrolein.

Throughout the studies, the bulk chemicals were stored in glass bottles at approximately 5° C (allyl acetate, acrolein) or room temperature (allyl alcohol). Reanalysis of bulk allyl acetate was performed by the study laboratory using high-performance liquid chromatography (HPLC) with an Inertsil ODS-2,150 mm × 4.6 mm, 5-µm column (Metachem Technologies) with ultraviolet detection at 210 nm and a solvent system of Milli-Q water:acetonitrile (70:30) at an isocratic flow rate of 0.7 mL/minute. Reanalyses of bulk allyl alcohol and acrolein were performed by the study laboratory using gas chromatography as described for the purity analysis of acrolein. Results indicated no degradation of the bulk chemicals.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing the chemical with 0.5% aqueous methylcellulose to form a suspension (allyl acetate) or solution (allyl alcohol and acrolein) (Table H1). Allyl acetate formulations were mixed with an overhead or magnetic stirrer; allyl alcohol was mixed by a magnetic stirrer or by shaking; and acrolein was mixed by inversion of the flask. All dose formulations were stored in amber glass bottles sealed with Teflon®-lined septa and refrigerated at approximately 5° C.

Homogeneity and stability studies of the 0.8 and 20 mg/mL allyl acetate formulations and stability studies of 0.6 and 10 mg/mL allyl alcohol formulations and 0.125 and 2 mg/mL acrolein formulations were performed by the study laboratory. Allyl acetate formulations were analyzed with HPLC using the same system described for the purity analysis of allyl acetate. The stability of the allyl alcohol and acrolein formulations was analyzed with gas chromatography using the same system described for the purity analysis of acrolein. Homogeneity was confirmed, and the stability of the allyl acetate and allyl alcohol dose formulations was confirmed for at least 21 days (allyl acetate) or 35 days (allyl alcohol) at room temperature or 5° C when stored sealed and protected from light. The stability of the acrolein formulations was confirmed for 7 days (0.125 mg/mL) or 14 days (2 mg/mL) at 5° C when stored sealed and protected from light.

Periodic analyses of the dose formulations were conducted by the study laboratory using HPLC (allyl acetate) or gas chromatography as described for the bulk purity analyses (Tables H2, H3, and H4). Dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples were also analyzed at most time points. All allyl acetate formulations were within 10% of the target concentrations except one rat formulation; this formulation, which was 11% greater than the target concentration, was used for dosing. All but two animal room samples for rats and four for mice were more than 10% below the target concentrations. In the allyl alcohol studies, 11 of 15 dose formulations for rats and 10 of 15 for mice were within 10% of the target concentrations; all dose formulations that were not within specifications were remixed and were found to be within 10% of the target concentrations. Nine of ten animal room samples of these dose formulations for rats and mice were within 10% of the target concentrations. Additionally, frozen samples of allyl alcohol formulations prepared on March 7, 1995, were analyzed concomitantly with animal room samples of the same dose formulations; all dose formulations were 11% to 28% less than the target concentrations. This was considered to be due to the large headspace in the storage vials, because the animal room samples were only 2% to 13% less than the target concentrations. All acrolein formulations for rats and mice were within 10% of the target concentrations; 7 of 15 animal room samples for rats and all but one for mice were more than 10% below the target concentrations. The chemical losses shown by the animal room sample analyses, particularly for the lower doses, were thought to be related to the volatility of the three chemicals.

TABLE H1
Preparation and Storage of Dose Formulations in the 14-Week Gavage Studies of Allyl Acetate, Allyl Alcohol, and Acrolein

Allyl Acetate	Allyl Alcohol	Acrolein
Preparation		
Allyl acetate was added to 0.5% aqueous methylcellulose and mixed with an overhead or magnetic stirrer to form a suspension.	Allyl alcohol was added to 0.5% aqueous methylcellulose and mixed with a magnetic stirrer or shaken to form a solution.	Same as allyl alcohol studies except mixed by inversion of the flask
Chemical Lot Number		
04525EF	00501TF	11163AG
Maximum Storage Time		
14 days	35 days	10 days
Storage Conditions		
Stored in amber glass bottles with Teflon®-lined septa at approximately 5° C	Stored in amber glass bottles with Teflon®-lined septa at approximately 5° C	Stored in amber glass bottles with Teflon®-lined septa at approximately 5° C
Study Laboratory		
Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)

TABLE H2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Allyl Acetate

Date Prepared	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats			
January 20, 1995	1.2	1.25	+4
	2.4	2.66	+11
	5	5.11	+2
	10	10.3	+3
	20	21.0	+5
March 6, 1995	1.2	1.16	–3
	2.4	2.32	–3
	5	5.20	+4
	10	9.82	–2
March 6, 1995[b]	1.2	0.978	–18
	2.4	2.00	–17
	5	4.30	–14
	10	7.71	–23
April 17, 1995	1.2	1.16	–3
	2.4	2.38	–1
	5	4.99	0
	10	9.95	0
April 17, 1995[b]	1.2	1.04	–13
	2.4	2.10	–12
	5	4.56	–9
	10	9.04	–10
Mice			
January 20, 1995	0.8	0.800	0
	1.6	1.71	+7
	3.2	3.43	+7
	6.25	6.54	+5
	12.5	13.1	+5
March 6, 1995	1.6	1.52	–5
	3.2	3.13	–2
	6.25	6.16	–1
March 6, 1995[b]	0.8	0.614	–23
	1.6	1.22	–24
	3.2	2.67	–17
	6.25	5.11	–18

TABLE H2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Allyl Acetate

Date Prepared	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)			
April 17, 1995	0.8	0.765	–4
	1.6	1.55	–3
	3.2	3.21	0
	6.25	6.20	–1
April 17, 1995[b]	0.8	0.725	–9
	1.6	1.48	–7
	3.2	3.07	–4
	6.25	6.11	–2

[a] Results of duplicate analyses. Dosing volume for rats=5 mL/kg; 1.2 mg/mL=6 mg/kg; 2.4 mg/mL=12 mg/kg; 5 mg/mL=25 mg/kg; 10 mg/mL=50 mg/kg; 20 mg/mL=100 mg/kg. Dosing volume for mice=10 mL/kg; 0.8 mg/mL=8 mg/kg; 1.6 mg/mL=16 mg/kg; 3.2 mg/mL=32 mg/kg; 6.25 mg/mL=62.5 mg/kg; 12.5 mg/mL=125 mg/kg.

[b] Animal room samples

TABLE H3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Allyl Alcohol

Date Prepared	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats			
February 7, 1995	0.3	0.307	+2
	0.6	0.574	–4
	1.2	1.20	0
	2.4	2.25	–6
	5	5.13	+3
March 7, 1995[b]	0.3	0.215	–28
	0.6	0.472	–21
	1.2	0.916	–24
	2.4	1.77	–26
	5	4.44	–11
March 7, 1995[c]	0.3	0.260	–13
	0.6	0.545	–9
	1.2	1.13	–6
	2.4	2.33	–3
	5	4.88	–2
April 5, 1995[d]	0.3	0.250	–17
	0.6	0.469	–22
	1.2	1.03	–16
	2.4	2.21	–8
	5	4.15	–17
April 7, 1995[e]	0.3	0.276	–8
	0.6	0.549	–8
	1.2	1.14	–5
	2.4	2.16	–10
	5	4.97	–1
April 7, 1995[c]	0.3	0.264	–12
	0.6	0.541	–10
	1.2	1.19	–1
	2.4	2.18	–9
	5	5.17	+3
May 2, 1995	0.3	0.294	–2
	0.6	0.573	–4
	1.2	1.15	–4
	2.4	2.27	–5
	5	4.81	–4
May 2, 1995[c]	0.3	0.309	+3
	0.6	0.615	+3
	1.2	1.22	+2
	2.4	2.37	–1
	5	4.89	–2

TABLE H3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Allyl Alcohol

Date Prepared	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice			
February 7, 1995	0.3	0.307	+2
	0.6	0.574	–4
	1.2	1.20	0
	2.5	2.52	+1
	5	5.13	+3
March 7, 1995[b]	0.3	0.215	–28
	0.6	0.472	–21
	1.2	0.916	–24
	2.5	2.08	–17
	5	4.44	–11
March 7, 1995[c]	2.5	2.41	–4
April 5, 1995[d]	0.3	0.250	–17
	0.6	0.469	–22
	1.2	1.03	–16
	2.5	2.18	–13
	5	4.15	–17
April 7, 1995[e]	0.3	0.276	–8
	0.6	0.549	–8
	1.2	1.14	–5
	2.5	2.45	–2
	5	4.97	–1
April 7, 1995[c]	0.3	0.263	–12
	0.6	0.547	–9
	1.2	1.15	–4
	2.5	2.31	–8
	5	5.25	+5
May 2, 1995	0.3	0.294	–2
	0.6	0.573	–4
	1.2	1.15	–4
	2.5	2.40	–4
	5	4.81	–4
May 2, 1995[c]	0.3	0.295	–2
	0.6	0.630	+5
	1.2	1.20	0
	2.5	2.45	–2
	5	4.90	–2

[a] Results of duplicate analyses. Dosing volume for rats=5 mL/kg; 0.3 mg/mL=1.5 mg/kg; 0.6 mg/mL=3 mg/kg; 1.2 mg/mL=6 mg/kg; 2.4 mg/mL=12 mg/kg=; 5 mg/mL=25 mg/kg. Dosing volume for mice=10 mL/kg; 0.3 mg/mL=3 mg/kg; 0.6 mg/mL=6 mg/kg; 1.2 mg/mL=12 mg/kg; 2.5 mg/mL=25 mg/kg; 5 mg/mL=50 mg/kg.
[b] Results for frozen archive samples
[c] Animal room samples
[d] Remixed; not used in study
[e] Results of remix

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Acrolein

Date Prepared	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats			
March 14, 1995	0.15	0.150	0
	0.25	0.249	0
	0.5	0.457	–9
	1	0.928	–7
	2	1.84	–8
March 14, 1995[b]	0.15	0.131	–13
	0.25	0.235	–6
	0.5	0.470	–6
	1	0.950	–5
	2	1.88	–6
April 20, 1995	0.15	0.146	–3
	0.25	0.250	0
	0.5	0.492	–2
	1	1.02	+2
	2	1.97	–1
April 20, 1995[b]	0.15	0.095	–37
	0.25	0.219	–12
	0.5	0.410	–18
	1	0.884	–12
	2	1.78	–11
June 12, 1995	0.15	0.146	–3
	0.25	0.254	+2
	0.5	0.475	–5
	1	0.948	–5
	2	1.90	–5
June 12, 1995[b]	0.15	0.145	–3
	0.25	0.251	0
	0.5	0.453	–9
	1	0.880	–12
	2	1.86	–7
Mice			
March 14, 1995	0.125	0.126	+1
	0.25	0.249	0
	0.5	0.457	–9
	1	0.928	–7
	2	1.84	–8
March 14, 1995[b]	0.125	0.108	–14
	0.25	0.218	–13
	0.5	0.438	–12
	1	0.883	–12
	2	1.74	–13

TABLE H4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Acrolein

Date Prepared	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)			
April 20, 1995	0.125	0.119	–6
	0.25	0.250	0
	0.5	0.492	–2
	1	1.02	+2
	2	1.97	–1
April 20, 1995[b]	0.125	0.080	–36
	0.25	0.218	–13
	0.5	0.421	–16
	1	0.895	–10
June 12, 1995	0.125	0.124	–1
	0.25	0.254	+2
	0.5	0.475	–5
	1	0.948	–5
	2	1.90	–5

[a] Results of duplicate analyses. Dosing volume for rats=5 mL/kg; 0.15 mg/mL=0.75 mg/kg; 0.25 mg/mL=1.25 mg/kg; 0.5 mg/mL=2.5 mg/kg; 1 mg/mL=5 mg/kg; 2 mg/mL=10 mg/kg. Dosing volume for mice=10 mL/kg; 0.125 mg/mL=1.25 mg/kg; 0.25 mg/mL=2.5 mg/kg; 0.5 mg/mL=5 mg/kg; 1 mg/mL=10 mg/kg; 2 mg/mL=20 mg/kg.
[b] Animal room samples

National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
P.O. Box 12233, MD K2-05
Durham, NC 27709
Tel: 984-287-3211
ntpwebrequest@niehs.nih.gov

https://ntp.niehs.nih.gov

ISSN 2378-8992