NTP REPORT

ON THE

TOXICOLOGY STUDIES OF ALLYL BROMIDE
(CAS NO. 106-95-6)

IN GENETICALLY MODIFIED (FVB Tg.AC HEMIZYGOUS) MICE

AND CARCINOGENICITY STUDIES OF ALLYL BROMIDE

IN GENETICALLY MODIFIED [B6.129-*Trp53*^{tm1Brd} (N5) HAPLOINSUFFICIENT] MICE

(DERMAL AND GAVAGE STUDIES)

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

April 2008

NTP GMM 7

NIH Publication No. 08-4424

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 Genetically Modified Model (GMM) Report series began in 2005 with studies conducted by the NTP. The studies described in the GMM Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected agents in laboratory animals that have been genetically modified. These genetic modifications may involve inactivation of selected tumor suppressor functions or activation of oncogenes that are commonly observed in human cancers. This may result in a rapid onset of cancer in the genetically modified animal when exposure is to agents that act directly or indirectly on the affected pathway. An absence of a carcinogenic response may reflect either an absence of carcinogenic potential of the agent or that the selected model does not harbor the appropriate genetic modification to reduce tumor latency and allow detection of carcinogenic activity under the conditions of these subchronic studies. Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP GMM 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 carcinogenic 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 GMM 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.

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CONTENTS

Summary

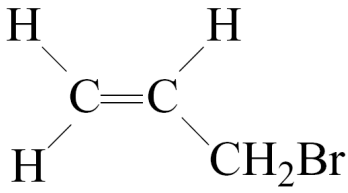
Background
Allyl bromide is used in the manufacture of polymers and resins, synthetic perfumes, pharmaceuticals, and agricultural products. We tested if allyl bromide could cause cancer in two different strains of genetically modified mice.

Methods
We gave solutions containing allyl bromide dissolved in corn oil to male and female Tg.AC hemizygous mice and to male and female p53 haploinsufficient mice by depositing the solution directly into their stomachs through a tube five days a week for 40 weeks. For each of the four studies, animals were given either 0.5, 1, 2, 4 or 8 mg of allyl bromide per kilogram of body weight; there were 15 animals per each dose group. Other groups of animals receiving only corn oil served as controls. Tissues from over 30 organs were examined for every animal.

Results
Some female Tg.AC hemizygous mice given allyl bromide developed squamous cell papillomas in the vulvar area. Male Tg.AC hemizygous mice and male and female p53 haploinsufficient mice receiving allyl bromide did not have any increase in tumors related to the chemical.

Conclusions
We conclude that allyl bromide may have caused a small increase in papillomas of the skin in female Tg.AC hemizygous mice but did not cause an increase in tumors in male Tg.AC hemizygous mice or in male or female p53 haploinsufficient mice.

ABSTRACT



ALLYL BROMIDE

CAS No. 106-95-6

Chemical Formula: C_3H_5Br Molecular Weight: 120.99

Synonyms: AB; 3-bromopropene; 3-bromopropylene; propene; 3-bromo-; 2-propenyl bromide

Allyl bromide is primarily used as a starting material/chemical intermediate in organic synthesis and as an intermediate in the manufacture of polymers/resins, synthetic perfumes, pharmaceuticals, agricultural chemicals, and other allyl compounds. It has been described as an insecticidal fumigant used in crop protection. Male and female FVB/N and C57BL/6 mice received allyl bromide (greater than 99% pure) by gavage and dermal application, respectively, for 2 weeks, and FVB/N, C57BL/6, Tg.AC hemizygous, and p53 haploinsufficient mice received allyl bromide by gavage for 40 weeks. Genetic toxicology studies were conducted in *Salmonella typhimurium* and mouse peripheral blood erythrocytes.

2-Week Study in FVB/N Mice

Groups of five male and five female FVB/N mice were dermally administered 0, 7.5, 15, 30, 60, or 120 mg allyl bromide/kg body weight in acetone, 5 days a week for 2 weeks. The survival and mean body weights of all dosed groups of males and females were similar to those of the vehicle controls. There were no increases in the incidences of lesions in dosed mice.

2-Week Study in C57BL/6 Mice

Groups of five male and five female C57BL/6 mice were administered 0, 7.5, 15, 30, 60, or 120 mg allyl bromide/kg body weight in corn oil by gavage, 5 days a week for 2 weeks. Three 120 mg/kg male mice died prior to the end of the study. Mean body weights of all dosed groups of males and females were similar to those of the vehicle controls. Liver weights of 30 and 60 mg/kg males were significantly greater than those of the vehicle controls. Nonneoplastic lesions of the forestomach, including hyperplasia, inflammation, degeneration, and hyperkeratosis of the forestomach epithelium, were observed in dosed mice.

40-Week Study in FVB/N Mice

Groups of 15 male and 15 female FVB/N mice were administered 0 or 8 mg allyl bromide/kg body weight in corn oil by gavage, 5 days a week for 40 weeks. Survival of dosed mice was similar to that of the vehicle controls. Mean body weights of dosed mice were within 10% of those of the vehicle controls throughout most of the study. There were no chemical-related gross or microscopic findings in dosed mice.

40-Week Study in Tg.AC Hemizygous Mice

Groups of 15 male and 15 female Tg.AC hemizygous mice were administered 0, 0.5, 1, 2, 4, or 8 mg allyl bromide/kg body weight in corn oil by gavage, 5 days a week for 40 weeks. Survival of dosed mice was similar to that of the vehicle controls. Mean body weights were generally similar between dosed and vehicle control mice throughout the study. In female mice, there were increased numbers of cutaneous and mucocutaneous masses (gross observations) on the body, particularly the vaginal and vulvar area, and these papillomas were observed earlier in the dosed groups. There were positive trends in the incidences of squamous cell papilloma of the vulva and of all skin sites in females.

40-Week Study in C57BL/6 Mice

Groups of 15 male and 15 female C57BL/6 mice were administered 0 or 8 mg allyl bromide/kg body weight in corn oil by gavage, 5 days a week for 40 weeks. Survival of dosed mice was similar to that of the vehicle controls. Mean body weights and organ weights were similar between dosed and vehicle control mice throughout the study. There were no chemical-related gross or microscopic findings in dosed mice.

40-Week Study in p53 Haploinsufficient Mice

Groups of 15 male and 15 female p53 haploinsufficient mice were administered 0, 0.5, 1, 2, 4, or 8 mg allyl bromide/kg body weight in corn oil by gavage, 5 days a week for 40 weeks. Survival of dosed mice was similar to that of the vehicle controls. Mean body weights of dosed mice were within 10% of those of the vehicle controls throughout most of the study. Mean body weights of 8 mg/kg females were 11% to 15% greater than those of the vehicle controls from week 26 to week 33, and those of 4 mg/kg females were generally less after week 21. There were no chemical-related gross or microscopic findings.

Genetic Toxicology

Allyl bromide was mutagenic in *S. typhimurium* strain TA100, with and without exogenous metabolic activation (S9). No mutagenicity was detected in *S. typhimurium* strain TA98, with or without S9, over the same concentration range tested with TA100. The frequency of micronucleated erythrocytes was assessed in male and female mice for each of the four mouse strains administered allyl bromide by corn oil gavage for 40 weeks. Results in all four micronucleus studies with allyl bromide were concluded to be negative; in addition, no significant changes in the percentage of polychromatic erythrocytes (reticulocytes) among total erythrocytes were observed in any of the four strains of mice.

Conclusions

Under the conditions of this study, there was *no evidence of carcinogenic activity** in male or female p53 haploinsufficient mice administered allyl bromide at 0.5, 1, 2, 4, or 8 mg/kg per day by corn oil gavage, 5 days a week for 40 weeks.

There was a marginal increase in the incidence of squamous cell papillomas, primarily of the vulva, in female Tg.AC hemizygous mice administered allyl bromide by corn oil gavage for 40 weeks. No treatment-related neoplasms were seen in male Tg.AC hemizygous mice administered allyl bromide by gavage at 0.5, 1, 2, 4, or 8 mg/kg, 5 days per week for 40 weeks.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Report appears on page 11.

Summary of the 40-Week Gavage and Genetic Toxicology Studies of Allyl Bromide in FVB/N Mice

	Male	Female
Concentrations in corn oil	0 or 8 mg/kg	0 or 8 mg/kg
Body weights	Dosed group similar to the vehicle control group	Dosed group similar to the vehicle control group
Survival rates	15/15, 14/15	15/15, 14/15
Nonneoplastic effects	None	None
Neoplastic effects	None	None
Genetic toxicology		
Salmonella typhimurium gene mutations:	Positive with and without S9 in TA100; negative with and without S9 in TA98	
Micronucleated erythrocytes		
Mouse peripheral blood *in vivo*:	Negative in males and females	

Summary of the 40-Week Gavage and Genetic Toxicology Studies of Allyl Bromide in Tg.AC Hemizygous Mice

	Male	Female
Concentrations in corn oil	0, 0.5, 1, 2, 4, or 8 mg/kg	0, 0.5, 1, 2, 4, or 8 mg/kg
Body weights	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group
Survival rates	12/15, 9/15, 9/15, 12/15, 6/15, 11/15	9/15, 10/15, 8/15, 8/15, 11/15, 12/15
Nonneoplastic effects	None	None
Neoplastic effects	None	Skin, vulva: squamous cell papilloma (2/15, 4/15, 1/15, 6/15, 5/14, 7/15) Skin, all sites: squamous cell papilloma (4/15, 6/15, 3/15, 7/15, 8/14, 9/15)
Genetic toxicology		
Salmonella typhimurium gene mutations:	Positive with and without S9 in TA100; negative with and without S9 in TA98	
Micronucleated erythrocytes		
Mouse peripheral blood *in vivo*:	Negative in males and females	

Summary of the 40-Week Gavage and Genetic Toxicology Studies of Allyl Bromide in C57BL/6 Mice

	Male	Female
Concentrations in corn oil	0 or 8 mg/kg	0 or 8 mg/kg
Body weights	Dosed group similar to the vehicle control group	Dosed group similar to the vehicle control group
Survival rates	14/15, 15/15	15/15, 12/15
Nonneoplastic effects	None	None
Neoplastic effects	None	None
Genetic toxicology		
Salmonella typhimurium gene mutations:	Positive with and without S9 in TA100; negative with and without S9 in TA98	
Micronucleated erythrocytes		
Mouse peripheral blood *in vivo*:	Negative in males and females	

Summary of the 40-Week Gavage and Genetic Toxicology Studies of Allyl Bromide in p53 Haploinsufficient Mice

	Male	Female
Concentrations in corn oil	0, 0.5, 1, 2, 4, or 8 mg/kg	0, 0.5, 1, 2, 4, or 8 mg/kg
Body weights	0.5, 4, and 8 mg/kg groups greater (generally within 10%) than the vehicle control group	8 mg/kg group greater (generally within 10%) than the vehicle control group; 4 mg/kg group less (generally within 10%) than the vehicle control group
Survival rates	15/15, 14/15, 15/15, 15/15, 13/15, 15/15	13/15, 14/15, 13/15, 14/15, 15/15, 13/15
Nonneoplastic effects	None	None
Neoplastic effects	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence
Genetic toxicology		
Salmonella typhimurium gene mutations:	Positive with and without S9 in TA100; negative with and without S9 in TA98	
Micronucleated erythrocytes		
Mouse peripheral blood *in vivo*:	Negative in males and females	

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence);** one category for uncertain findings **(equivocal evidence);** one category for no observable effects **(no evidence);** and one category for experiments that cannot be evaluated because of major flaws **(inadequate study).** These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Report on allyl bromide on August 28, 2006, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On August 28, 2006, the draft Report on the toxicology and carcinogenesis studies of allyl bromide received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.E. French, NIEHS, provided an overview of the development of the genetically modified mouse models used in the studies being reported. Dr. J.K. Dunnick, NIEHS, introduced the studies of allyl bromide in p53 haploinsufficient and Tg.AC hemizygous mice by describing the uses of the chemical, the study rationale, the details of the study design and dose selection, and the results of the histopathologic examination of the animals. The proposed conclusions were: *no evidence of carcinogenic activity* in male or female p53 haploinsufficient mice administered allyl bromide at 0.5, 1, 2, 4, or 8 mg/kg per day by corn oil gavage, 5 days a week for 40 weeks. There was a marginal increase in the incidence of squamous cell papillomas, primarily of the vulva, in female Tg.AC hemizygous mice administered allyl bromide by corn oil gavage for 40 weeks.

Dr. Giesy, the first principal reviewer, did not have any scientific criticisms, felt the report presented the results clearly, and agreed with the proposed conclusions.

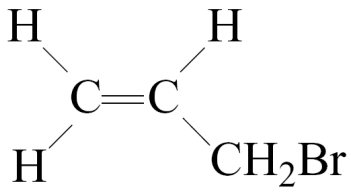
Dr. Mirsalis, the second principal reviewer, questioned the decision to perform a gavage study in the FVB/N mice at doses that showed no effect in a pilot dermal study. He suggested more explanation of the rationale for design of that study. He felt the other studies were valid and agreed with the proposed conclusions.

Dr. Sikka, the third principal reviewer, suggested additions to the metabolic pathway diagram and asked for an explanation of why the chemical was mutagenic in the absence, but not the presence, of metabolic activation.

Dr. Dunnick replied that the discussions of mutagenicity and the descriptions of study design would be amplified. She noted that oral gavage was the route of choice for all the genetically modified mouse model studies.

Dr. Mirsalis moved, and Dr. Giesy seconded, that the conclusions be accepted as written. The motion was approved unanimously with seven votes.

INTRODUCTION



ALLYL BROMIDE

CAS No. 106-95-6

Chemical Formula: C_3H_5Br Molecular Weight: 120.99

Synonyms: AB; 3-bromopropene; 3-bromopropylene; propene; 3-bromo-; 2-propenyl bromide

Chemical and Physical Properties

Allyl bromide is a colorless to light yellow liquid with an unpleasant pungent odor; the boiling point at 760 mm Hg is 71.3° C; the melting point is –119° C, and the density/specific gravity is 1.398 at 4° C (*Merck*, 1996). The octanol/water partition coefficient (log P) is given as 1.59 by Lipnik *et al.* (1987) and 1.79 by Hansch *et al.* (1995). Allyl bromide is slightly soluble in water and miscible with alcohol, chloroform, ether, carbon disulfide, or carbon tetrachloride (*Merck*, 1996). The allyl bromide water solubility is 3,835 mg/L at 25° C (Yalkowsky and Dannenfelser, 1992). Allyl bromide may react with water with some release of energy, but not violently (NFPA, 1997). Dangerous fire and explosion hazards occur when allyl bromide is exposed to heat, flame, or oxidizers; it also emits toxic bromide fumes when heated to decomposition (Lewis, 1997).

Production, Use, and Human Exposure

Allyl bromide is produced by a reaction of hydrogen bromide and allyl alcohol; from a reaction of hydrobromic acid and allyl alcohol; or from a reaction of triphenylphosphite, allyl alcohol, and benzyl bromide (*Merck*, 1996).

Allyl bromide is used primarily as a starting material/chemical intermediate in organic synthesis and as an intermediate in the manufacture of polymers/resins, synthetic perfumes, pharmaceuticals, agricultural chemicals (Kirino *et al.,* 1980; Kim *et al.,* 1992), and other allyl compounds (Stenger, 1978; *Merck*, 1996). It has also been described as an insecticidal fumigant used in crop protection (Stenger, 1978; Gosselin *et al.*, 1984). The United States Environmental Protection Agency (2002) reported that the production volume for allyl bromide in 1998 and 2002 was between 10,000 and 500,000 pounds. Nineteen United States suppliers were listed in the Chemical Buyers' Guide for 2000 [personal communication from V. Fung, National Cancer Institute (NCI)]. Occupational exposure to allyl bromide may occur through dermal contact, inhalation, or ingestion (HSDB, 2003).

Allyl bromide is not known to occur naturally. However, allyl bromide has been identified as a pyrolytic degradation product of brominated polymers (Grassie *et al.*,

1986). Allyl bromide has also been identified as a water and air pollutant. Bauman and Stenstrom (1989) identified allyl bromide as one of a group of halogen compounds in seven sources of wastewater and drinking water. Allyl bromide is a major photolytic degradation product of aqueous 1,2-dibromopropene in the absence and presence of hydrogen peroxide (Milano and Vernet, 1988). The allyl bromide yield was 25% relative to the initial trace amount of 1,2-dibromopropene present as a water contaminant. Allyl bromide was one of 78 toxic, volatile, organic compounds routinely monitored (Dunn *et al.*, 1987). In a Russian study, Zenkevich and Konyukhova (1992) reported that allyl bromide was an ecologically significant contaminant.

Regulatory Status

Allyl bromide is listed in Section 8b of the U.S. Environmental Protection Agency's Toxic Substances Control Act Chemical Substances Inventory (USEPA, 2006). The National Fire Protection Association (1997) hazard classification is a grade 3; on short exposure, allyl bromide could cause serious injury, and full protective clothing including self-contained breathing apparatus is recommended. The state of Pennsylvania lists allyl bromide as a hazardous substance and tracks it as a potential workplace hazard (Shafer, 1995). It is regulated by the United States Department of Transportation as a flammable liquid (STN, 1994; Shafer, 1995).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Kaye *et al.* (1972) studied allyl halide metabolism in male rats. After the animals were treated subcutaneously with 0.5 mL allyl bromide, 3-hydroxypropylmercapturic acid and allylmercapturic acid and its sulfoxide were excreted in the urine. According to the authors, these metabolites can be formed by a number of different pathways because allyl halides can undergo reactions at either the double bond or the halide bonding site. Eder *et al.* (1986) performed metabolism studies on allyl compounds, including allyl bromide, as part of a screening strategy to investigate genotoxic potential. They reported that allylic compounds, which are alkylating agents, are detoxified via substitution reactions with glutathione (GSH) to produce mercapturic acids. In addition, allyl bromide undergoes metabolic transformation to acrolein as a reactive intermediate; however, it does not appear to be metabolized via an epoxide route. The metabolic pathways proposed for allyl bromide are shown schematically in Figure 1.

Humans

No information on the absorption, distribution, metabolism, or excretion of allyl bromide in humans was found in the literature.

Toxicity

Experimental Animals

The oral LD_{50} of allyl bromide in rats is 120 mg/kg; the intraperitoneal LD_{50} in mice is 108 mg/kg (Lewis, 1996). Lipnick *et al.* (1987) reported an LC_{50} for goldfish of 0.8 mg/mL.

The Hazardous Substance Data Bank (HSDB, 2003) reported a toxicity study of allyl bromide conducted by Shell Oil Company in Wistar rats. In this study, allyl bromide was administered by gavage to 10 male rats per group for 14 days at 15 or 60 mg/kg body weight. Additional groups were exposed to control vehicles (water or arachis oil). At 60 mg/kg, the compound caused gastric irritation and reduced body weight gain.

No other toxicity studies of allyl bromide were reported in the literature.

Humans

No epidemiological studies or reports of health effects in humans related to exposure to allyl bromide were found in the literature.

Reproductive Toxicity

In the study reported by the HSDB (2003), Shell Oil Company evaluated testicular toxicity in Wistar rats. Wistar rats were exposed orally to 0, 15, or 60 mg allyl bromide/kg body weight per day for 14 days. On day 15, there were no treatment-related changes in the morphology of the kidney, testes, epididymides, ductuli efferentes, or vasa deferentes or in testes weights.

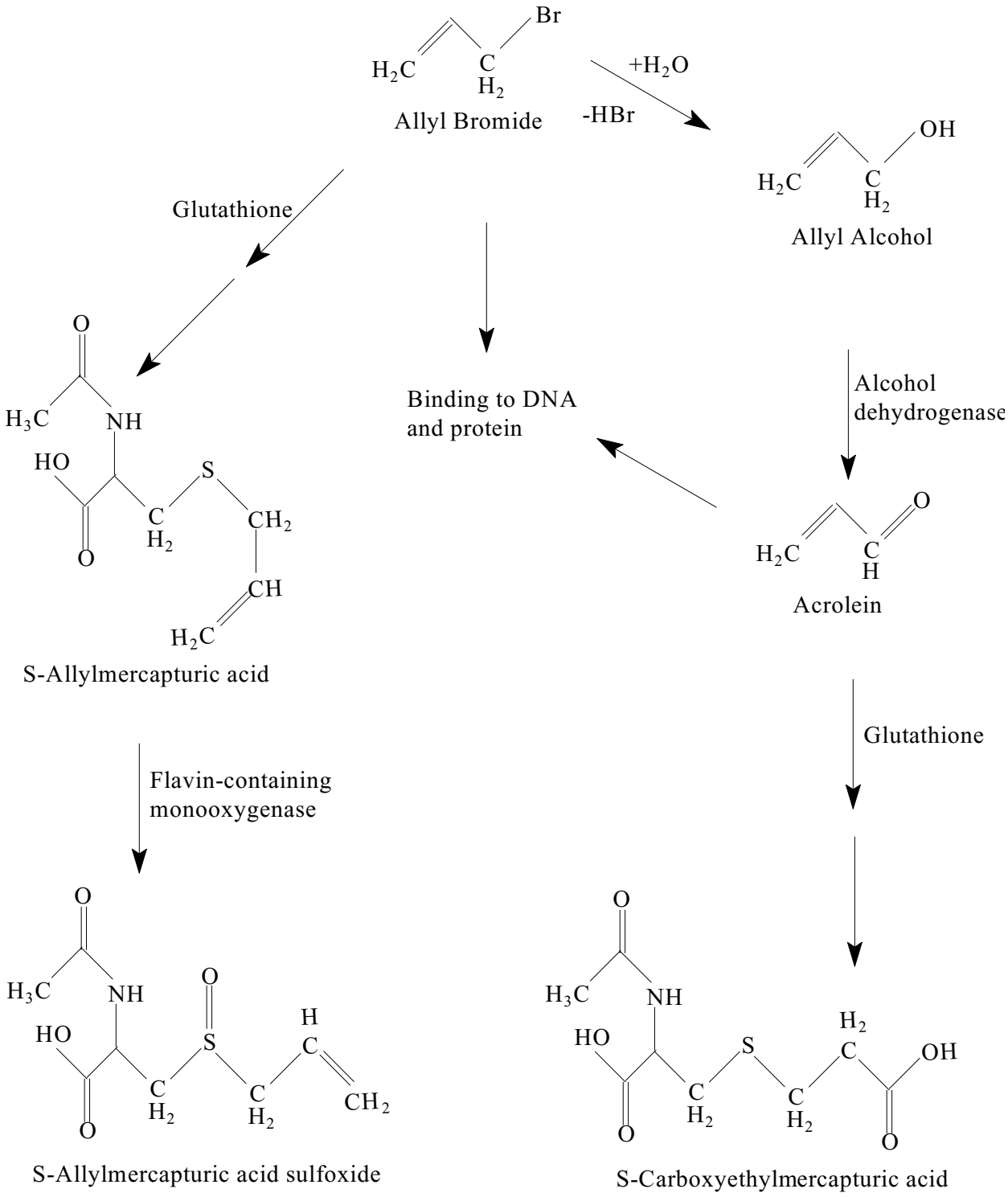


FIGURE 1
Metabolic Pathways for Allyl Bromide (modified from Eder *et al.*, 1986 and Krause *et al.*, 2002)

CARCINOGENICITY

Experimental Animals

Allyl bromide is structurally related to allyl chloride. In the NCI (1977) bioassay of allyl chloride, the chemical was negative for carcinogenic activity in male and female rats and equivocal in male and female mice. There are no 2-year carcinogenicity studies of allyl bromide.

Humans

No epidemiological studies of allyl bromide were found in the literature.

GENETIC TOXICITY

Allyl bromide is a reactive electrophile and direct-acting alkylating agent, and it has been shown to bind to DNA in *in vitro* model systems (Eder *et al.*, 1982, 1987; Eder and Zugelder, 1990; Ashby and Paton, 1993). Allyl bromide (100% pure) was reported to be mutagenic in the absence of S9 activation in *Salmonella typhimurium* strain TA100 under conditions that controlled for volatility (Eder *et al.*, 1980). Lijinsky and Andrews (1980) also reported allyl bromide to be mutagenic in *S. typhimurium* strain TA100 in the absence of metabolic activation; the addition of S9 reduces the mutagenic activity of allyl bromide.

Schiffmann *et al.* (1983) reported that allyl bromide induced a dose-dependent increase in unscheduled DNA synthesis (UDS) in HeLa S3 cells. Eder *et al.* (1980) reported direct correlations among mutagenic potency in *S. typhimurium* strain TA100 (expressed as revertants/μM), alkylating ability, and activity in the UDS assay with several allyl halides, including allyl bromide. Furthermore, comparison of three allyl halides in the UDS assay gave the following activity levels: allyl iodide > allyl bromide > allyl chloride (Eder *et al.*, 1983). Consistent with other studies, Schiffmann *et al.* (1983) reported a direct correlation between activity in the UDS assay and mutagenicity in *Salmonella* for these same three allyl halides. In contrast, allylic compounds with greater UDS activity than allyl bromide but lower mutagenicity include *cis*- and *trans*-1,3-dichloropropene, 1-chloro-2-butene, and 2,3-dichloro-1-propene. Thus, the correlation between mutagenicity in *Salmonella* and activity in the UDS assay is not consistent among all allylic compounds.

Studies of *in vitro* binding of allyl bromide, allyl methanesulphonate, and allyl chloride to salmon sperm DNA (Eder *et al.,* 1987) indicated that all three allyl compounds bound to DNA yielding the same five allyl substituted nucleic bases: N^2-allylguanine, O^6-allylguanine, N^7-allylguanine, N^3-allyladenine, and N^6-allyladenine. The order of potency was allyl methanesulphonate > allyl bromide > allyl chloride. The *in vitro* binding half-life for allyl bromide was 8.1 hours at 37° C (Eder *et al.,* 1986). In an *in vivo* DNA binding study in which ^{14}C-labeled allyl bromide was administered by gavage to mice, these same five allylated nucleic bases were identified in hydrolysate DNA from different organs indicating direct DNA reactivity for allyl bromide in the whole animal (Eder *et al.*, 1983, 1986). Using isolated rat liver perfused with solutions containing either allyl bromide or allyl chloride, Eder and Zugelder (1990) again demonstrated the *in vivo* formation of the five adducts described above. The authors suggested that the formation of allyl adducts, especially the promutagenic O^6-guanine adduct, clearly indicates cancer-initiating potential.

Two allyl bromide structural analogs, allyl chloride and 3-chloro-2-methylpropene, have been tested for mutagenicity by the National Toxicology Program (NTP). Allyl chloride, like allyl bromide, was mutagenic in *S. typhimurium* strain TA100 in the absence of S9 (unpublished data). 3-Chloro-2-methylpropene (methyl allyl chloride), which was more extensively tested for mutagenicity, produced positive results in a variety of assays. It was mutagenic in *S. typhimurium* strain TA1537 with S9, but in contrast to allyl bromide and allyl chloride, it was negative in strain TA100, with and without S9 (Haworth *et al.*, 1983; Zeiger *et al.*, 1988). In addition, 3-chloro-2-methylpropene was mutagenic in cultured mammalian cells without S9 (Myhr and Caspary, 1991), and it induced chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary (CHO) cells in the absence of S9 (Gulati *et al.*, 1989). Positive results were also obtained in a sex-linked recessive lethal mutation assay in *Drosophila melanogaster* (Foureman *et al.*, 1994). Despite these positive results for induced chromosomal damage and mutagenicity in a number of test systems, 3-chloro-2-methylpropene did not induce micronucleated reticulocytes in bone marrow of male mice treated by intraperitoneal injection with up

to 250 mg/kg (Shelby *et al.*, 1993). Thus, none of these allylic compounds, all of which are *in vitro* mutagens, have been shown to induce effects *in vivo*.

In rats, the metabolic pathway for allyl bromide and allyl chloride was shown to produce S-carboxyl mercapturic acid, which is the principal metabolite of acrolein; neither compound is metabolized via an epoxide pathway (Eder *et al.,* 1987). The mutagenic activity of acrolein has been well studied and appears to be inconsistently detected among a variety of *in vitro* assays (NTP, 2006), probably due to its extreme electrophilicity, allowing it to react readily with a variety of nucleophilic compounds (Beauchamp *et al.*, 1985). Acrolein has demonstrated direct DNA alkylation (Henschler and Eder, 1986; Foiles *et al.*, 1990; Eder *et al.*, 1993) and is 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.*, 1996). It also induced sister chromatid exchanges but not chromosomal aberrations in cultured CHO cells (Galloway *et al.*, 1987). Conflicting results have been reported for *in vitro* mammalian cell mutagenicity assays (Curren *et al.*, 1988). Like allyl bromide, acrolein does not increase the frequency of micronucleated erythrocytes in mice treated with the compound for 3 months. The *in vivo* metabolism of allyl bromide and allyl chloride likely also involves direct alkylation of GSH, resulting in the formation of nonmutagenic mercapturic acids.

Background on Genetically Altered Mice

Mutation and/or deletions of tumor suppressor genes or activation of protooncogenes can disrupt cell function and predispose an animal to cancer. In the current studies, two genetically altered mouse models with either a loss of heterozygosity in a critical cancer gene (Trp53) or a gain of oncogene function (Ha *ras*) were used to determine how these animals would respond to allyl bromide exposure. These mouse models are susceptible to the rapid development of cancer. The Tg.AC hemizygous and p53 haploinsufficient mice are being evaluated by the National Institute of Environmental Health Sciences (NIEHS) and the NTP as models for identifying chemical toxicity and/or chemical carcinogenic processes (Tennant *et al*., 1996; Pritchard *et al*., 2003).

FVB/N-TgN(v-Ha-ras)Led (Tg.AC) Hemizygous Mouse Model

Tg.AC mice are hemizygous for a mutant v-Ha-*ras* transgene. The Tg.AC mouse (on an FVB/N background) was developed by Leder *et al*. (1990) by introduction via pronuclear injection of a tripartite transgene composed of the promoter of the mouse embryonic zeta-globin gene, through the v-Ha-*ras* coding sequence, with point mutation in codons 12 and 59, and a simian virus 40 polyadenylation sequence. Because the inducible zeta-globin promoter drives the expression of a mutated v-Ha-*ras* oncogene, the Tg.AC mouse is regarded as a genetically initiated model.

The Tg.AC transgenic mouse model has been evaluated as a reporter phenotype (skin papillomas) in response to either genotoxic or nongenotoxic carcinogens, including tumor promoters (Spalding *et al*., 1993, 1999; Tennant *et al.*, 1999). With the exception of bone marrow, constitutive expression of the transgene cannot be detected in adult tissues. The transgene is transcriptionally silent until activated by certain treatments including full-thickness wounding, ultraviolet irradiation, or exposure to some chemicals (Cannon *et al.*, 1997; Trempus *et al*., 1998). The Tg.AC hemizygous mouse develops a high incidence of skin papillomas in response to topical application of 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), and TPA has been used as a positive control in NIEHS Tg.AC mouse studies (Spalding *et al.*, 1993). TPA has been used as a positive control in NIEHS Tg.AC mouse studies to confirm the mice are responsive to carcinogens because it has been found that the subset of Tg.AC mice may revert and become nonresponsive to a tumor promoter (Honchel *et al.*, 2001). Point mutations in the Ha-*ras* gene are believed to be early events in the induction of skin papillomas and malignancies. Topical application of carcinogens to the shaved dorsal surface of Tg.AC mice induces epidermal squamous cell papillomas or carcinomas, a reporter phenotype that defines the activity of the chemical. The oral route of administration can also generate tumor responses in the skin of Tg.AC mice and lead to squamous cell papillomas and/or carcinomas of the forestomach. To date, the appearance of either spontaneous or induced tumors has been

shown to involve transgene expression. However, the mechanism of response by the Tg.AC model to chemical carcinogens is not yet understood.

In NIEHS studies, mice are exposed beginning at 2 months of age for a total of 6 to 9 months. Cutaneous papillomas at various sites have been reported at 10% and 7% incidence in 33-week-old control male and female Tg.AC mice, respectively (Mahler *et al*., 1998). Cutaneous papillomas occurring at sites such as the lip, pinnae, prepuce, and vulva suggest a possible relationship to grooming and chronic irritation. Up to 32% of Tg.AC homozygous and heterozygous male or female mice can develop odontogenic tumors as early as 33 weeks (Wright *et al*., 1995; Mahler *et al*., 1998). A number of different tumor types occur in untreated Tg.AC hemizygous mice at an incidence of greater than 3% including odontogenic tumors, forestomach papillomas, cutaneous papillomas, alveolar/bronchiolar adenomas, salivary gland duct carcinomas, and erythroleukemia (Mahler *et al*., 1998). In the FVB mouse (the background strain for the Tg.AC hemizygous mouse), alveolar/bronchiolar neoplasms occur at 14 months of age (Mahler *et al*., 1996).

The Tg.AC hemizygous mouse model was used in the current report for the studies of allyl bromide because this model has been reported to detect both nongenotoxic and genotoxic carcinogens (Spalding *et al*., 1993; Tennant *et al*., 1995, 1996; Pritchard *et al*., 2003).

B6.129-Trp53^{tm1Brd} (N5) Haploinsufficient Mouse Model

The heterozygous B6.129-Trp53(N12)$^{tmlBrd(+/–)}$ mouse (on a B6.129S7 background) was developed by Donehower *et al*. (1992). A null mutation was introduced into one p53 allele by homologous recombination in murine embryonic stem cells. Insertion of a neo cassette resulted in deletion of a 450-base pair gene fragment containing 106 nucleotides of exon 5 and approximately 350 nucleotides of intron 4.

Trp53, a nuclear protein, plays an essential role in the regulation of the cell cycle, specifically in the transition from G_0 to G_1, as well as G_2 to M, and the spindle apparatus. The p53 protein has a short half-life and exists at very low concentration under normal cell physiological conditions. However, in DNA damaged cells that are able to replicate, p53 is expressed in high amounts with a significant increase in half-life due to post-translational modification (phosphorylation or acetylation). Mutation in p53 may also increase the protein half-life and alter the functions that may contribute to transformation and development of the malignant phenotype. p53 is a DNA-binding protein containing DNA-binding, oligomerization, and transcription activation domains. Many amino acid residues in different p53 domains may be phosphorylated or acetylated, which may determine specific p53 functions. It is postulated to bind as a tetramer to a p53-binding site and activate expression of downstream genes that inhibit growth and/or invasion or promote apoptosis, functioning as a tumor suppressor. This protein is critical to tumor suppression in humans and rodents. Mutants of p53 that fail to bind the consensus DNA binding site, and hence are unable to function as tumor suppressors, frequently occur in human cancers. Alterations of the Trp53 gene occur not only as somatic mutations in human malignancies, but also as germline mutations in some cancer-prone families with Li-Fraumeni syndrome.

The mouse heterozygous for a p53 null allele (+/–) has only a single functional wild-type p53 allele, which provides a target for mutagens. The p53 tumor suppressor gene is one of the most common sites for mutations and gene alterations in human cancer (Harris, 1996a,b,c).

Heterozygous p53 mice develop normally and, like humans and other mammals, develop cancer (primarily lymphomas or sarcomas) with age, but often with decreased latency.

Study Rationale

Allyl bromide was nominated for study by the NCI because there was no existing 2-year carcinogenicity study for the chemical. Transgenic mouse models were used to screen for toxicity and carcinogenicity. The p53 haploinsufficient and Tg.AC hemizygous mouse models were selected for this screen because they were the models under study at the NTP for possible use in chemical hazard identification.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Allyl Bromide

Allyl bromide was obtained from Fluka Chemical Corporation (Buchs, Switzerland) in one lot (330638) and from Aldrich Chemical Co. in one lot (03614HN). Lot 330638 was used in the 2-week studies, and lot 03614HN was used in the 40-week studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) and the study laboratory, BioReliance (Rockville, MD). Reports on analyses performed in support of the allyl bromide studies are on file at the National Institute of Environmental Health Sciences.

Both lots of allyl bromide, a clear, colorless liquid, were identified by the analytical chemistry laboratory using infrared and proton nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using infrared spectroscopy. All infrared and NMR spectra were consistent with the literature spectra and spectra of a reference standard from the same lot. The purity of each lot was determined by the analytical chemistry and study laboratories using gas chromatography (GC). For lot 330638, GC indicated one major peak and five impurities with a combined peak area of 0.7% relative to the total peak area. GC by a second system indicated one major peak and three impurities with a combined peak area of less than 0.5%. The relative purity was 102% when compared to a reference standard from the same lot. The overall purity of lot 330638 was greater than 99%. For lot 03614HN, GC indicated one major peak and four impurities with a combined peak area of 0.45% relative to the total peak area. GC by a second system indicated one major peak and three impurities with a total combined area less than 0.3% of the total peak area. The relative purity was 102% when compared to a frozen reference from the same lot. The overall purity of lot 03614HN was greater than 99%. During the 40-week studies, additional purity analyses were performed by the study laboratory at 26 weeks and at the end of the study using GC.

To ensure stability, the bulk chemical was stored in a sealed container under a nitrogen headspace, protected from light, at 2° to 8° C. No degradation of the bulk chemical was detected.

Acetone

ACS-grade acetone was obtained from Fisher Scientific (Hampton, NH) in two lots (963514 and 982335) that were used as the vehicle in the 2-week dermal study. The study laboratory determined the identity using infrared spectroscopy and the purity using GC. Infrared spectra were consistent with a literature spectrum. GC indicated a major peak; two impurities of 0.15% and 0.05% of the total peak area; several minor impurities, each less than 0.01% of the total peak area; and an overall purity greater than 99.7%.

Corn Oil

Corn oil in multiple lots was used as the vehicle during the 2-week and 40-week gavage studies. The study laboratory analyzed peroxide levels prior to use and monthly during the study using potentiometric titration; all peroxide concentrations were less than 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

For the 2-week dermal study, the dose formulations were prepared once by pipetting the appropriate amounts of allyl bromide and acetone into a volumetric flask and mixing thoroughly (Table G2). The dose formulations were stored in amber glass vials under a headspace of inert gas, protected from light, at 2° to 8° C for at least 35 days.

Prior to the 2-week dermal study, the analytical chemistry laboratory conducted stability studies on 1 mg/mL formulations of allyl bromide in acetone using GC. Formulations were stored in glass vials capped with Teflon®-lined septa, protected from light, at 25° and

5° C, and at simulated animal room conditions. Stability was confirmed for at least 35 days at 25° and 5° C and for at least 3 hours at animal room conditions.

For the 2-week and 40-week gavage studies, the appropriate amounts of allyl bromide and corn oil were pipetted into a volumetric flask and mixed thoroughly. Dose formulations were prepared once for the 2-week study and every 2 weeks during the 40-week studies. Dose formulations were stored in amber glass vials with Teflon®-lined septa and aluminum crimp caps under a headspace of inert gas, protected from light, at 2° to 8° C for up to 21 days, with the exception of formulations used between November 16, 1999, and December 20, 1999, which were stored for 27 days. Dose formulations prepared on December 7, 1999, stored at 2° to 8° C for 28 days, then at –20° C until analyzed on January 13, 2000, confirmed stability for up to 28 days (Table G3).

A solubility study of allyl bromide in corn oil was conducted by the analytical chemistry laboratory using GC; the maximum solubility was 142.2 mg/mL. No homogeneity studies were conducted on dose formulations in corn oil as concentrations used in the 2-week study (0.75 to 12.0 mg/mL) and 40-week studies (0.05 to 0.80 mg/mL) were well below the maximum solubility.

For the 2-week and 40-week gavage studies, the analytical chemistry laboratory conducted stability studies on 0.37 mg/mL formulations of allyl bromide in corn oil using GC. Formulations were stored in amber glass vials capped with Teflon®-lined septa, protected from light, at 25° and 5° C, and at simulated animal room conditions. Stability was confirmed for at least 16 days at 25° C, at least 21 days at 5° C, and at least 3 hours at animal room conditions. Later, a second stability study was conducted by the analytical chemistry laboratory on 0.74 mg/mL formulations under the same conditions as those previously described. No significant trend toward loss was observed at 25° or 5° C for at least 42 days, though variability was large (RSD 10.8%), and no significant loss was observed at animal room conditions for at least 3 hours.

Periodic analyses of the dose formulations were conducted by the study laboratory using GC. For the 2-week dermal and gavage studies, the dose formulations were analyzed once. Animal room samples were also analyzed. Of the dose formulations used and analyzed for the dermal study, all five were within 10% of the target concentrations; all five animal room samples were within 10% of target concentrations (Table G4). Of the dose formulations used and analyzed for the 2-week gavage study, all five were within 10% of the target concentrations; one of five animal room samples was within 10% of the target concentrations (Table G5). For the 40-week gavage studies, dose formulations were analyzed at least every 12 weeks; animal room samples were also analyzed. Of the dose formulations used and analyzed, all 25 were within 10% of the target concentrations; 17 of 30 animal room samples were within 10% of the target concentrations (Table G3).

STUDY DESIGNS

Dose Selection Rationale

The 2-week studies were conducted in the parent strains of the Tg.AC hemizygous and p53 haploinsufficient mice, which were FVB/N and C57BL/6 mice, respectively. Allyl bromide was administered by gavage and dermal routes of administration to C57BL/6 and FVB/N mice, respectively. The gavage route was selected because the National Toxicology Program (NTP) had considered the gastrointestinal tract to be the target organ in previous studies of other brominated chemicals (NTP, 2006). Because of the limited water solubility of allyl bromide, corn oil was used as the gavage vehicle. The dermal route was used to mimic potential workplace exposure. The doses for the 2-week studies were based on an oral LD_{50} in rats of 120 mg/kg. No oral LD_{50} for mice was reported in the literature (RTECS, 2002). The intraperitoneal LD_{50} for mice was reported to be 108 mg/kg (Lewis, 1996). The dose concentrations selected for the 2-week studies were 0, 7.5, 15, 30, 60, and 120 mg/kg for gavage doses of allyl bromide (in corn oil) to C57BL/6 mice and dermal doses of allyl bromide (in acetone) to FVB/N mice.

2-Week Dermal Study

Groups of five male and five female FVB/N mice received dermal applications of 0, 7.5, 15, 30, 60, or 120 mg allyl bromide/kg body weight in 3.3 mL acetone/kg body weight, 5 days per week for 16 days. Vehicle control mice were administered acetone only. Doses were applied to the clipped dorsal skin from the mid-back to the interscapular area.

2-Week Gavage Study

Groups of five male and five female C57BL/6 mice received 0, 7.5, 15, 30, 60, or 120 mg allyl bromide/kg body weight in 10 mL corn oil/kg body weight by gavage, 5 days per week for 16 days. Vehicle control mice received corn oil only.

40-Week Gavage Studies

Groups of 15 male and 15 female FVB/N and C57BL/6 mice received 0 or 8 mg allyl bromide/kg body weight in corn oil by gavage, in a volume of 10 mL/kg body weight, 5 days per week for 40 weeks. Groups of 15 male and 15 female Tg.AC hemizygous and p53 haploinsufficient mice received 0, 0.5, 1, 2, 4, or 8 mg allyl bromide/kg body weight in 10 mL/kg corn oil by gavage, 5 days per week for 40 weeks. Vehicle control mice received corn oil only.

Positive Control Mice

Positive control groups of 15 male and 15 female Tg.AC hemizygous mice received dermal applications of 1.25 µg TPA in 100 µL acetone (12.5 µg TPA/L solution), three times per week until removal from study. Positive control mice were removed after the appearance of 20 or more skin papillomas and discarded. The TPA solution was applied to the clipped dorsal skin from the mid-back to the interscapular area.

Source and Specification of Animals

Male and female FVB/N, C57BL/6, Tg.AC hemizygous, and p53 haploinsufficient mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-week and 40-week studies. FVB/N and C57BL/6 mice were quarantined for 14 or 15 days, respectively, before the beginning of the 2-week studies. FVB/N and Tg.AC hemizygous mice were quarantined for 11 or 12 days and C57BL/6 and p53 haploinsufficient mice were quarantined for 12 days before the beginning of the 40-week studies. Before the 2-week and 40-week studies, five male and five female mice per strain were randomly selected for parasite evaluations and gross observations of disease. FVB/N and C57BL/6 mice were 7 or 8 weeks old, respectively, at the beginning of the 2-week studies. FVB/N, Tg.AC hemizygous, C57BL/6, and p53 haploinsufficient mice were 7, 6, 9, and 9 weeks old, respectively, at the beginning of the 40-week studies. At the end of the 40-week studies, blood samples were collected from the retroorbital sinus of five male and five female vehicle control mice per strain. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al*., 1989a,b). All results were negative.

Animal Maintenance

Mice were housed individually. Feed and water were available *ad libitum*. Cages and racks were rotated every other week during the 40-week studies. Further details of animal maintenance are given in Table 1.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded daily during the 2-week studies and weekly during the 40-week studies. Clinical findings were recorded postdosing. Body weights were recorded initially, weekly, and at the end of the studies.

Necropsies and microscopic examinations were performed on all mice except positive controls. The heart, right kidney, liver, lung, right testis, and thymus were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. Upon completion of the laboratory pathologists' histopathologic evaluations, 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 evaluated by an independent quality assessment laboratory; few discrepancies were identified. The NTP pathologist reviewed the lesions, and consensus diagnosis was achieved among the original pathologist, the quality assurance pathologist, and the NTP pathologist for all neoplastic and skin lesions. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

TABLE 1
Experimental Design and Materials and Methods in the Dermal and Gavage Studies of Allyl Bromide

2-Week Dermal Study	2-Week Gavage Study	40-Week Gavage Studies
Study Laboratory		
BioReliance (Rockville, MD)	BioReliance (Rockville, MD)	BioReliance (Rockville, MD)
Strain and Species		
FVB/N mice	C57BL/6 mice	FVB/N mice C57BL/6 mice FVB/N-TgN(v-Ha-*ras*)(Tg.AC) hemizygous mice B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient mice
Animal Source		
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
14 days	15 days	FVB/N and Tg.AC mice: 11-12 days C57BL/6 and p53 mice: 21 days
Average Age When Studies Began		
7 weeks	8 weeks	FVB/N: 7 weeks Tg.AC: 6 weeks C57BL/6 and p53 mice: 9 weeks
Date of First Dose		
September 15, 1998	September 16, 1998	FVB/N and Tg.AC mice: March 22, 1999 C57BL/6 and p53 mice: April 1, 1999
Duration of Dosing		
16 days	16 days	40 weeks
Date of Last Dose		
September 30, 1998	October 1, 1998	FVB/N mice: December 22, 1999 C57BL/6 mice: January 4, 2000 Tg.AC mice: December 21, 1999 p53 mice: January 3, 2000
Necropsy Dates		
October 1, 1998	October 2, 1998	FVB/N mice: December 23, 1999 C57BL/6 mice: January 5, 2000 Tg.AC mice: December 21-22, 1999 p53 mice: December 30, 1999-January 4, 2000
Average Age at Necropsy		
10 weeks	10 weeks	FVB/N mice: 47 weeks Tg.AC mice: 45 weeks C57BL/6 mice: 49 weeks p53 mice: 48-49 weeks
Size of Study Groups		
5 males and 5 females	5 males and 5 females	15 males and 15 females

TABLE 1
Experimental Design and Materials and Methods in the Dermal and Gavage Studies of Allyl Bromide

2-Week Dermal Study	2-Week Gavage Study	40-Week Gavage Studies
Animals per Cage		
1	1	1
Method of Animal Identification		
Tail tattoo	Tail tattoo	Tail tattoo
Diet		
Irradiated NTP-2000 Open Formula Diet/ pelleted form (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as dermal study	Same as dermal study
Water		
Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as dermal study	Same as dermal study
Cages		
Polycarbonate (Lab Products, Inc., Seaford, DE), changed weekly	Same as dermal study	Same as dermal study
Bedding		
Irradiated heat-treated, Sani-Chip hardwood bedding (P.J. Murphy Forest Products, Montville, NJ), changed weekly	Same as dermal study	Same as dermal study
Cage Filters		
Remay 2016 (Snow Filtration, West Chester, OH), changed weekly	Same as dermal study	Same as dermal study except changed every other week
Racks		
Stainless Steel (Lab Products, Inc., Seaford, DE), changed weekly	Same as dermal study	Same as dermal study, rotated every other week
Animal Room Environment		
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses		
0, 7.5, 15, 30, 60, or 120 mg/kg allyl bromide in acetone at a volume of 3.3 mL/kg body weight 5 days per week	0, 7.5, 15, 30, 60, or 120 mg/kg allyl bromide in corn oil at a volume of 10 mL/kg body weight, 5 days per week	FVB/N and C57BL/6 mice: 0 or 8 mg/ kg allyl bromide in corn oil by gavage at a volume of 10 mL/kg body weight, 5 days per week Tg.AC and p53 mice: 0, 0.5, 1, 2, 4, or 8 mg/kg allyl bromide in corn oil by gavage at a volume of 10 mL/kg body weight, 5 days per week, or 1.25 µg TPA applied dermally in 100 µL acetone three times/week (Tg.AC positive controls only)

TABLE 1
Experimental Design and Materials and Methods in the Dermal and Gavage Studies of Allyl Bromide

2-Week Dermal Study	2-Week Gavage Study	40-Week Gavage Studies
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as dermal study	Same as dermal study
Type and Frequency of Observation		
Observed twice daily; animals were weighed initially, day 8, and at the end of the study; clinical findings were recorded daily.	Same as dermal study	Observed twice daily; animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly.
Method of Sacrifice		
CO_2 asphyxiation	CO_2 asphyxiation	CO_2 asphyxiation
Necropsy		
Necropsies were performed on all animals. Organs weighed were heart, liver, lungs, right kidney, right testis, and thymus.	Same as dermal study	Necropsies were performed on all animals (except positive controls). Organs weighed were heart, liver, lungs, right kidney, right testis, and thymus.
Histopathology		
Histopathology was performed on all animals. In addition to gross lesions and tissue masses, the skin (treated and untreated sites) and stomach (forestomach and glandular) were examined.	Histopathology was performed on all animals. In addition to gross lesions and tissue masses, the stomach (forestomach and glandular) was examined.	Histopathology was performed on all animals (except positive controls). In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular, mediastinal, and mesenteric), ovary, pituitary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, and uterus.

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Animals found dead of other than natural causes or missing were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendixes A, B, C, and D as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test (Gart *et al.,* 1979) and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.,* 1979), procedures based on the overall proportion of affected animals, were used to determine significance.

Analysis of Continuous Variables

Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

QUALITY ASSURANCE METHODS

The 40-week studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from these studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Report.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1992). Allyl bromide was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98 and TA100 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 37º C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of allyl bromide. The high dose was limited by toxicity. All trials that gave a positive response were repeated.

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

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990) and Witt *et al.* (2000). At the end of the 40-week studies, peripheral blood samples were obtained from male and female mice from each strain. 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 up to 15 mice per group. In addition, the percentage of polychromatic erythrocytes (PCEs) in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials. 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. Because these studies were not repeated, the results of the single micronucleus trials in these mice were accepted without replication.

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 summary tables in the Abstract of this Report present a result that represents a scientific judgment of the overall evidence for activity of the chemical in an assay.

RESULTS

2-WEEK DERMAL STUDY IN FVB/N MICE

All animals survived to the end of the study (Table 2). Final group mean body weights and body weight gains of dosed males and females were similar to those of the vehicle control groups (Table 2). No chemical-related clinical findings were observed.

There were no significant differences in organ weights in male or female mice treated with allyl bromide when compared to their respective vehicle controls (Table F1). No chemical-related gross observations were noted at necropsy. Under microscopic observation, one female in the group administered 120 mg/kg had a hyperplasia of the skin at the site of application.

TABLE 2
Survival and Body Weights of FVB/N Mice in the 2-Week Dermal Study of Allyl Bromide

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	24.7 ± 0.8	27.1 ± 0.9	2.4 ± 0.6	
7.5	5/5	24.6 ± 0.8	27.2 ± 0.9	2.5 ± 0.3	100
15	5/5	24.6 ± 0.4	27.0 ± 0.2	2.4 ± 0.3	100
30	5/5	25.4 ± 0.6	27.2 ± 0.4	1.8 ± 0.4	100
60	5/5	24.0 ± 1.0	27.6 ± 0.5	3.6 ± 1.4	102
120	5/5	25.1 ± 0.4	27.5 ± 0.9	2.4 ± 0.9	101
Female					
0	5/5	19.6 ± 0.6	21.5 ± 0.5	2.0 ± 0.2	
7.5	5/5	19.1 ± 0.5	21.9 ± 0.5	2.8 ± 0.3	102
15	5/5	19.3 ± 0.2	20.9 ± 0.4	1.6 ± 0.2	97
30	5/5	19.2 ± 0.4	21.3 ± 0.4	2.1 ± 0.5	99
60	5/5	19.8 ± 0.6	21.8 ± 0.3	2.0 ± 0.4	101
120	5/5	19.5 ± 0.7	21.8 ± 0.5	2.3 ± 0.3	101

[a] Number of animals surviving at 17 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the vehicle control groups are not significant by Dunnett's test.

2-Week Gavage Study in C57BL/6 Mice

Three 120 mg/kg male mice died prior to the terminal sacrifice (Table 3). All female mice survived to the end of the study. Final mean body weights and body weight gains of dosed mice were similar to those of the vehicle control groups (Table 3). Lethargy was observed in one male each in the 60 and 120 mg/kg groups and in two females in the 120 mg/kg group.

Absolute and relative liver weights of 30 and 60 mg/kg males were significantly greater than those of the vehicle control group (Table F4). Absolute and relative testis weights of 60 mg/kg males and absolute and relative heart weights of 120 mg/kg females were significantly less.

Dosed mice developed nonneoplastic lesions in the forestomach (Table 4). Treatment caused severe, necrotizing, and ulcerative gastritis, occasionally with transmural ulcers, in male and female mice, particularly at high doses. At necropsy, adhesions of the stomach serosa to surrounding organs (liver and spleen) and abdominal wall were evident in 40% to 100% of the mice in the 60 and 120 mg/kg groups (data not shown). These findings are consistent with peritonitis associated with degeneration, necrosis, and ulceration observed in all dosed groups leading to transmural, gastric ulcers in some mice in the 120 mg/kg groups. There were also treatment-related increases in the incidences of gastric epithelial hyperplasia and inflammation.

Table 3
Survival and Body Weights of C57BL/6 Mice in the 2-Week Gavage Study of Allyl Bromide

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	21.0 ± 1.3	22.2 ± 0.9	1.2 ± 1.2	
7.5	5/5	21.4 ± 0.9	23.1 ± 0.6	1.7 ± 0.9	104
15	5/5	21.8 ± 0.8	23.2 ± 1.0	1.4 ± 0.4	104
30	5/5	22.2 ± 0.5	23.5 ± 0.9	1.3 ± 0.5	106
60	5/5	21.7 ± 0.9	23.1 ± 0.8	1.4 ± 0.3	104
120	2/5[c]	21.1 ± 1.4	22.2 ± 0.4	0.0 ± 0.0	100
Female					
0	5/5	17.1 ± 0.3	19.8 ± 0.5	2.7 ± 0.6	
7.5	5/5	17.7 ± 0.6	19.2 ± 1.1	1.5 ± 1.0	97
15	5/5	17.3 ± 0.5	20.0 ± 0.5	2.8 ± 0.4	101
30	5/5	17.5 ± 0.3	19.7 ± 0.7	2.3 ± 0.8	99
60	5/5	17.3 ± 0.5	18.7 ± 0.5	1.4 ± 0.6	94
120	5/5	17.1 ± 0.5	18.6 ± 0.8	1.5 ± 0.3	94

[a] Number of animals surviving at 17 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. Differences from the control group are not significant by Dunnett's test.
[c] Days of death: 7, 8, 13

TABLE 4
Nonneoplastic Forestomach Lesions in C57BL/6 Mice in the 2-Week Gavage Study of Allyl Bromide

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Male						
Number Examined Microscopically	5	5	5	5	5	5
Hyperkeratosis, Diffuse[a]	0	0	1	2	2	2
Hyperkeratosis, Focal	0	0	2	1	3	1
Epithelium, Degeneration, Diffuse	0	1	1	1	1	0
Epithelium, Degeneration, Focal	0	0	2	2	0	0
Epithelium, Hyperplasia, Diffuse	0	0	1	2	0	1
Epithelium, Hyperplasia, Focal	0	0	2	1	4*	2
Epithelium, Inflammation, Chronic Active, Focal	0	0	1	3	1	0
Epithelium, Ulcer, Focal	0	0	0	0	0	1
Female						
Number Examined Microscopically	5	5	5	5	5	5
Hyperkeratosis, Diffuse	0	2	0	2	0	1
Hyperkeratosis, Focal	0	0	0	0	5**	4*
Epithelium, Degeneration, Focal	0	1	4*	5**	0	0
Epithelium, Hyperplasia, Focal	0	3	4*	5**	5**	5**
Epithelium, Inflammation, Chronic Active, Focal	0	0	0	2	0	0
Epithelium, Necrosis, Focal	0	0	0	0	2	0
Muscularis, Serosa, Epithelium, Inflammation, Chronic Active, Focal	0	0	0	0	4*	0
Muscularis, Serosa, Epithelium, Ulcer, Focal	0	0	0	0	3	5**

* 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 lesion

Dose Selection Rationale: In the 2-week C57BL/6 mouse study, there were significant forestomach lesions at the 15, 30, 60, and 120 mg/kg concentrations. Because of this forestomach toxicity, a high dose of 8 mg/kg per day was selected for the 40-week study in p53 haploinsufficient mice. No toxicity was seen in the FVB/N mice in the 2-week dermal study. Thus, in the 40-week study in Tg.AC hemizygous mice, the oral route of administration was used at the same doses as in the 40-week study in p53 haploinsufficient mice. The parental strains (FVB/N and C57BL/6 mice) were exposed to 0 or 8 mg/kg by corn oil gavage in 40-week studies. In the allyl bromide studies in the p53 and Tg.AC mice, there was no evidence for tumor formation or decrease in survival at 26 weeks. Exposure was continued to 40 weeks to allow more time for the development of toxic or carcinogenic processes.

40-Week Gavage Study in FVB/N Mice

Survival

Estimates of 40-week survival probabilities for male and female mice are shown in Table 5. Survival of dosed male and female mice was similar to that of the vehicle control groups. One male and one female administered 8 mg/kg died before the end of the study.

Body Weights, Clinical Findings, and Organ Weights

Mean body weights of dosed male mice were generally similar to those of vehicle controls, and those of dosed female mice were generally greater throughout the study (Figure 2; Tables 6 and 7). There were no treatment-related clinical findings. Organ weights of 8 mg/kg males and females were similar to those of the vehicle control groups (Table F2).

Pathology and Statistical Analyses

There were no statistically or biologically significant increases in neoplasms or nonneoplastic lesions in FVB/N mice administered allyl bromide by gavage for 40 weeks. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Appendix A for male and female FVB/N mice. Three (20%) dosed males (8 mg/kg), two (13%) vehicle control females, and one (7%) dosed female (8 mg/kg) developed alveolar/bronchiolar adenomas (Tables A1 and A2). Mahler *et al.* (1996) reported spontaneous alveolar/bronchiolar adenoma rates in 14-month-old FVB/N mice of 2/45 (4%) for males and 8/98 (8%) for females. Mice in the current study were approximately 11 months old at the end of the study.

Table 5
Survival of FVB/N Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	8 mg/kg
Male		
Animals initially in study	15	15
Accidental death[a]	0	1
Animals surviving to study termination	15	14
Percent probability of survival at end of study[b]	100	100
Mean survival (days)[c]	277	259
Survival analysis[d]		—[e]
Female		
Animals initially in study	15	15
Moribund	0	1
Animals surviving to study termination	15	14
Percent probability of survival at end of study	100	93
Mean survival (days)	276	272
Survival analysis		P=1.000

[a] Censored from survival analysis
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group column.
[e] Value of statistic cannot be computed.

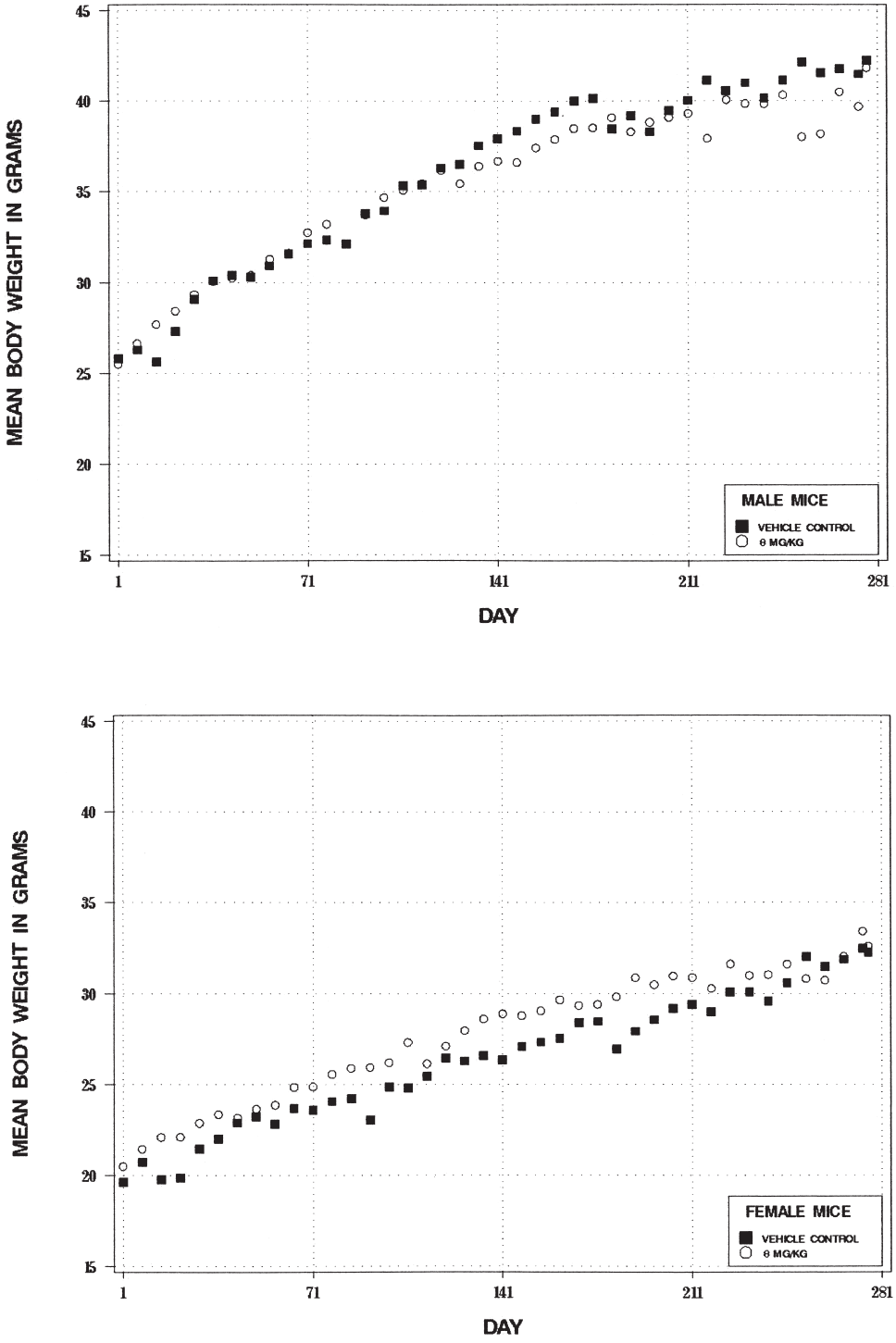


FIGURE 2
Growth Curves for Male and Female FVB/N Mice Exposed to Allyl Bromide by Gavage for 40 Weeks

TABLE 6
Mean Body Weights and Survival of Male FVB/N Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	Vehicle Control		8 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	25.8	15	25.5	99	15
2	26.3	15	26.6	101	14
3	25.6	15	27.7	108	14
4	27.3	15	28.4	104	14
5	29.1	15	29.3	101	14
6	30.1	15	30.1	100	14
7	30.4	15	30.2	99	14
8	30.3	15	30.4	100	14
9	30.9	15	31.3	101	14
10	31.6	15	31.6	100	14
11	32.1	15	32.7	102	14
12	32.3	15	33.2	103	14
13	32.1	15	32.1	100	14
14	33.8	15	33.7	100	14
15	34.0	15	34.7	102	14
16	35.3	15	35.1	99	14
17	35.4	15	35.4	100	14
18	36.3	15	36.2	100	14
19	36.5	15	35.4	97	14
20	37.5	15	36.4	97	14
21	37.9	15	36.7	97	14
22	38.4	15	36.6	95	14
23	39.0	15	37.4	96	14
24	39.4	15	37.9	96	14
25	40.0	15	38.5	96	14
26	40.2	15	38.5	96	14
27	38.5	15	39.1	102	14
28	39.2	15	38.3	98	14
29	38.3	15	38.8	101	14
30	39.5	15	39.1	99	14
31	40.0	15	39.3	98	14
32	41.2	15	37.9	92	14
33	40.6	15	40.1	99	14
34	41.0	15	39.8	97	14
35	40.2	15	39.8	99	14
36	41.1	15	40.3	98	14
37	42.1	15	38.0	90	14
38	41.6	15	38.2	92	14
39	41.8	15	40.5	97	14
40	41.5	15	39.7	96	14
Mean for Weeks					
1-13	29.5		29.9	101	
14-40	38.9		37.8	97	

TABLE 7
Mean Body Weights and Survival of Female FVB/N Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	Vehicle Control		8 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.6	15	20.5	105	15
2	20.7	15	21.4	103	15
3	19.8	15	22.1	112	15
4	19.9	15	22.1	111	15
5	21.5	15	22.9	107	15
6	22.0	15	23.4	106	15
7	22.9	15	23.2	101	15
8	23.2	15	23.6	102	15
9	22.8	15	23.9	105	15
10	23.7	15	24.8	105	15
11	23.6	15	24.9	106	15
12	24.1	15	25.6	106	15
13	24.2	15	25.9	107	15
14	23.1	15	25.9	112	15
15	24.9	15	26.2	105	15
16	24.8	15	27.3	110	15
17	25.5	15	26.1	102	15
18	26.5	15	27.1	102	15
19	26.3	15	28.0	107	15
20	26.6	15	28.6	108	15
21	26.4	15	28.9	110	15
22	27.1	15	28.8	106	15
23	27.3	15	29.0	106	15
24	27.5	15	29.6	108	15
25	28.4	15	29.3	103	15
26	28.5	15	29.4	103	15
27	27.0	15	29.8	110	15
28	27.9	15	30.9	111	15
29	28.6	15	30.5	107	15
30	29.2	15	31.0	106	15
31	29.4	15	30.9	105	15
32	29.0	15	30.3	105	14
33	30.1	15	31.6	105	14
34	30.1	15	31.0	103	14
35	29.6	15	31.0	105	14
36	30.6	15	31.6	103	14
37	32.0	15	30.8	96	14
38	31.5	15	30.7	98	14
39	31.9	15	32.0	100	14
40	32.5	15	33.4	103	14
Mean for Weeks					
1-13	22.2		23.4	106	
14-40	28.2		29.6	105	

40-Week Gavage Study in Tg.AC Hemizygous Mice

Positive Control Tg.AC Hemizygous Mice

12-*O*-Tetradecanoylphorbol-13-acetate (1.25 µg) was dermally administered to groups of 15 male and 15 female mice. Except for one female that died early, all males and females developed more than 20 skin papillomas each by week 18 (data not shown). This is consistent with historical rates found in other studies and indicates that the Tg.AC mice in this entire study were of the "responder" genotype (Tennant *et al*., 2001).

Survival

Estimates of 40-week survival probabilities for male and female mice are shown in Table 8. Survival of dosed mice was similar to that of the vehicle control groups.

Body Weights, Clinical Findings, and Organ Weights

Mean body weights of dosed mice were generally similar to those of the vehicle control mice throughout the study (Figure 3; Tables 9 and 10). There were no treatment-related clinical findings in male mice. In female mice, there were increased numbers of cutaneous and mucocutaneous masses (gross observations) on the body, particularly the vaginal and vulvar area, and these papillomas were observed earlier in the dosed groups. There were no biologically significant differences in organ weights of dosed groups compared to the vehicle control groups (Table F3).

Table 8
Survival of Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Male						
Animals initially in study	15	15	15	15	15	15
Accidental deaths[a]	0	1	0	1	1	1
Moribund	2	2	3	2	4	1
Natural deaths	1	3	3	0	4	2
Animals surviving to study termination	12	9	9	12	6	11
Percent probability of survival at end of study[b]	80	64	60	86	43	79
Mean survival (days)[c]	264	228	227	241	213	233
Survival analysis[d]	P=1.000	P=0.480	P=0.304	P=1.000N	P=0.055	P=1.000
Female						
Animals initially in study	15	15	15	15	15	15
Accidental deaths[a]	2	0	0	0	0	0
Moribund	2	3	4	6	1	3
Natural deaths	2	2	3	1	3	0
Animals surviving to study termination	9	10	8	8	11	12
Percent probability of survival at end of study	71	67	53	53	73	80
Mean survival (days)	247	246	232	245	248	267
Survival analysis	P=0.265N	P=1.000	P=0.454	P=0.524	P=1.000N	P=0.816N

[a] Censored from survival analysis
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dosed group is indicated by **N**.

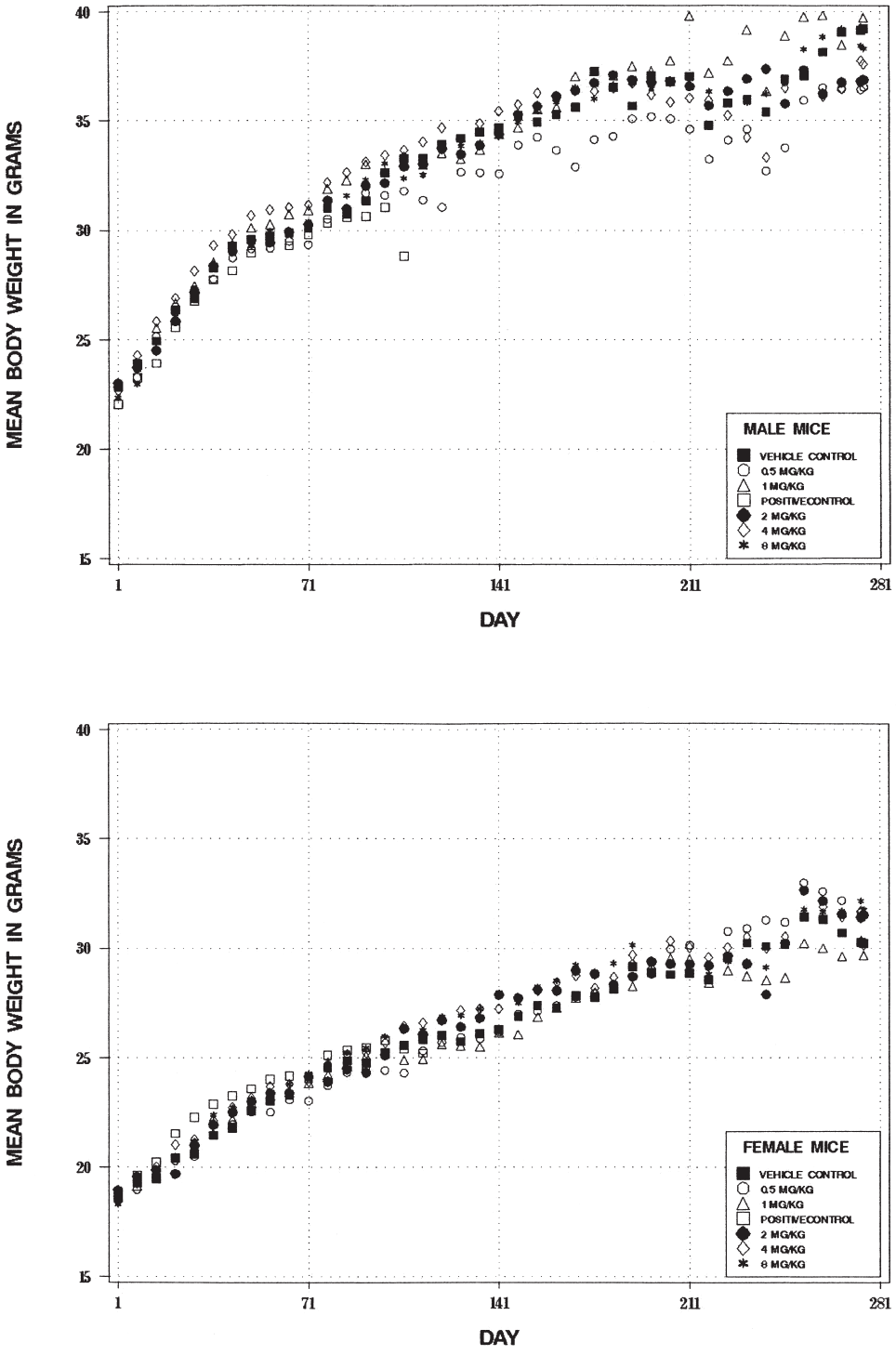


FIGURE 3
Growth Curves for Male and Female Tg.AC Hemizygous Mice Exposed to Allyl Bromide by Gavage for 40 Weeks

TABLE 9
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	Vehicle Control		0.5 mg/kg			1 mg/kg			2 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.8	15	22.9	100	15	23.0	101	15	23.0	101	15
2	23.9	15	23.3	98	15	24.0	100	15	23.7	99	15
3	24.9	15	25.1	101	14	25.5	102	15	24.5	98	14
4	26.4	15	26.5	100	14	26.7	101	15	25.8	98	14
5	26.9	15	27.0	100	14	27.5	102	15	27.2	101	14
6	28.3	15	27.8	98	14	28.5	101	15	28.4	100	14
7	29.3	15	28.7	98	14	29.4	100	15	29.0	99	14
8	29.6	15	29.1	98	14	30.1	102	15	29.5	100	14
9	29.7	15	29.2	98	14	30.3	102	15	29.4	99	14
10	29.9	15	29.5	99	14	30.7	103	15	29.9	100	14
11	30.1	15	29.3	97	14	30.9	103	14	30.3	101	14
12	31.0	15	30.5	98	14	31.9	103	14	31.4	101	14
13	30.7	15	31.0	101	14	32.3	105	14	31.0	101	14
14	31.3	15	31.7	101	14	33.0	105	14	32.0	102	14
15	32.6	15	31.6	97	14	32.6	100	14	32.1	99	14
16	33.3	15	31.8	96	14	33.4	100	14	32.9	99	14
17	33.3	15	31.4	94	14	33.0	99	14	33.0	99	14
18	33.9	15	31.0	91	14	33.5	99	13	33.7	99	14
19	34.2	15	32.6	95	14	33.3	97	13	33.5	98	14
20	34.5	15	32.6	95	14	33.7	98	13	33.9	98	14
21	34.7	15	32.6	94	14	34.4	99	13	34.4	99	14
22	35.2	15	33.9	96	14	34.7	99	13	35.3	100	13
23	34.9	15	34.2	98	14	35.5	102	12	35.7	102	13
24	35.3	15	33.6	95	14	35.6	101	12	36.1	102	13
25	35.6	15	32.9	92	12	37.0	104	11	36.4	102	12
26	37.3	14	34.1	91	12	37.2	100	11	36.7	98	12
27	36.5	14	34.3	94	12	37.0	101	11	37.1	102	12
28	35.7	14	35.1	98	11	37.5	105	11	36.9	103	12
29	37.1	14	35.2	95	11	37.3	101	11	36.8	99	12
30	36.8	14	35.1	95	11	37.7	102	11	36.8	100	12
31	37.0	14	34.6	94	10	39.8	108	10	36.6	99	12
32	34.8	14	33.2	95	10	37.2	107	10	35.7	103	12
33	35.8	14	34.1	95	9	37.7	105	10	36.4	102	12
34	36.0	14	34.6	96	9	39.1	109	9	36.9	103	12
35	35.4	14	32.7	92	9	36.3	103	9	37.4	106	12
36	36.9	12	33.8	92	9	38.9	105	9	35.8	97	12
37	37.1	12	35.9	97	9	39.8	107	9	37.3	101	12
38	38.1	12	36.5	96	9	39.8	105	9	36.2	95	12
39	39.1	12	36.4	93	9	38.5	99	9	36.8	94	12
40	39.1	12	36.4	93	9	39.2	100	9	36.8	94	12
Mean for Weeks											
1-13	28.0		27.7	99		28.5	102		27.9	100	
14-40	35.6		33.8	95		36.4	102		35.5	100	

TABLE 9
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	4 mg/kg			8 mg/kg		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.7	100	15	22.3	98	15
2	24.3	102	14	23.0	96	14
3	25.8	104	14	25.0	100	14
4	26.9	102	14	26.1	99	14
5	28.1	105	14	27.3	102	14
6	29.3	104	14	28.3	100	14
7	29.8	102	14	29.0	99	14
8	30.7	104	14	29.3	99	14
9	30.9	104	14	29.9	101	14
10	31.0	104	14	30.0	100	14
11	31.2	104	14	30.4	101	14
12	32.2	104	14	31.0	100	14
13	32.6	106	14	31.6	103	14
14	33.1	106	14	32.3	103	14
15	33.4	103	14	33.0	101	14
16	33.7	101	14	32.4	97	14
17	34.0	102	14	32.5	98	12
18	34.7	102	14	33.6	99	12
19	34.1	100	13	33.8	99	12
20	34.9	101	12	34.0	99	12
21	35.4	102	12	34.7	100	12
22	35.7	101	12	35.0	99	12
23	36.2	104	12	35.4	101	12
24	35.9	102	12	35.8	101	12
25	36.4	102	11	36.5	103	12
26	36.3	97	11	36.0	97	12
27	36.5	100	11	36.7	101	12
28	36.7	103	11	36.7	103	12
29	36.2	98	11	36.5	98	12
30	35.8	97	10	36.9	100	12
31	36.0	97	10	36.9	100	12
32	35.9	103	10	36.3	104	12
33	35.2	98	8	36.4	102	12
34	34.2	95	8	35.8	99	12
35	33.3	94	7	36.2	102	12
36	36.5	99	6	36.7	100	11
37	37.2	100	6	38.3	103	11
38	36.1	95	6	38.8	102	11
39	36.5	93	6	39.2	100	11
40	37.7	96	6	38.4	98	11
Mean for Weeks						
1-13	28.9	103		27.9	100	
14-40	35.5	100		35.7	100	

TABLE 10
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	Vehicle Control		0.5 mg/kg			1 mg/kg			2 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.6	15	18.5	100	15	18.9	102	15	18.9	102	15
2	19.3	15	19.0	98	15	19.2	100	15	19.5	101	15
3	19.5	15	19.5	100	15	19.9	102	15	19.9	102	15
4	20.4	15	20.3	100	15	20.4	100	15	19.7	97	15
5	20.6	15	20.5	100	15	20.9	102	15	21.0	102	15
6	21.5	15	21.5	100	15	22.0	102	15	21.9	102	15
7	21.8	15	21.9	101	15	22.2	102	15	22.5	103	15
8	22.6	15	22.5	100	15	22.9	101	15	23.0	102	15
9	23.0	15	22.5	98	15	23.3	101	15	23.4	102	15
10	23.3	15	23.1	99	15	23.4	100	15	23.4	100	15
11	24.1	15	23.0	95	15	23.8	99	15	24.1	100	15
12	24.5	15	23.7	97	15	24.2	99	15	23.9	98	15
13	24.9	15	24.3	98	15	24.7	99	15	24.5	98	15
14	24.8	15	24.5	99	15	25.1	101	15	24.3	98	15
15	25.2	15	24.4	97	15	25.2	100	15	25.1	100	15
16	25.6	15	24.3	95	15	24.9	97	14	26.3	103	15
17	25.8	15	25.3	98	15	24.9	97	14	26.1	101	15
18	26.0	15	25.7	99	15	25.6	99	14	26.7	103	15
19	25.7	15	25.9	101	15	25.5	99	14	26.4	103	15
20	26.1	15	25.9	99	15	25.5	98	14	26.8	103	15
21	26.3	15	26.2	100	15	26.2	100	14	27.9	106	14
22	26.9	15	27.0	100	14	26.1	97	14	27.7	103	14
23	27.4	13	27.1	99	14	26.9	98	13	28.1	103	14
24	27.3	13	27.4	100	14	27.3	100	13	28.1	103	14
25	27.8	13	27.8	100	13	27.7	100	12	29.0	104	14
26	27.7	13	27.9	101	13	28.0	101	12	28.8	104	14
27	28.1	13	28.2	100	13	28.2	100	12	28.3	101	14
28	29.1	13	29.3	101	13	28.3	97	12	28.7	99	14
29	28.9	13	28.9	100	13	29.2	101	10	29.4	102	12
30	28.8	13	30.0	104	12	29.5	102	10	29.3	102	12
31	28.9	13	30.1	104	12	29.5	102	10	29.3	101	12
32	28.5	11	29.2	103	12	28.4	100	10	29.2	103	11
33	29.5	11	30.8	104	10	29.0	98	10	29.6	100	11
34	30.2	10	30.9	102	10	28.7	95	10	29.3	97	11
35	30.1	10	31.3	104	10	28.5	95	9	27.9	93	11
36	30.2	10	31.2	103	10	28.6	95	9	30.2	100	9
37	31.4	10	33.0	105	10	30.2	96	8	32.6	104	8
38	31.3	10	32.6	104	10	30.0	96	8	32.2	103	8
39	30.7	9	32.2	105	10	29.6	96	8	31.6	103	8
40	30.2	9	31.6	105	10	30.4	101	8	31.4	104	8
Mean for Weeks											
1-13	21.9		21.6	99		22.0	101		22.0	101	
14-40	28.1		28.5	101		27.7	99		28.5	102	

TABLE 10
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	4 mg/kg			8 mg/kg		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.8	101	15	18.3	98	15
2	19.5	101	15	19.2	100	15
3	20.0	103	15	19.9	102	15
4	21.0	103	15	20.5	101	15
5	21.2	103	15	21.2	103	15
6	22.1	103	15	22.4	104	15
7	22.7	104	15	22.7	104	15
8	23.2	103	15	23.1	102	15
9	23.7	103	15	23.2	101	15
10	23.8	102	15	23.8	102	15
11	23.9	99	15	24.3	101	15
12	24.6	100	15	24.8	101	15
13	25.1	101	15	25.2	101	15
14	25.2	102	14	25.4	102	15
15	25.7	102	14	26.0	103	15
16	26.4	103	14	26.4	103	15
17	26.6	103	14	26.2	102	15
18	26.7	103	14	26.9	104	15
19	27.2	106	14	26.9	105	15
20	27.2	104	14	27.2	104	15
21	27.2	103	14	28.0	107	15
22	27.7	103	14	27.5	102	15
23	28.0	102	14	28.2	103	15
24	28.4	104	14	28.5	104	15
25	28.7	103	14	29.2	105	15
26	28.2	102	14	28.9	104	15
27	28.6	102	14	29.3	104	15
28	29.7	102	13	30.1	103	15
29	28.9	100	13	28.7	99	15
30	30.3	105	12	29.3	102	15
31	30.0	104	12	29.3	101	15
32	29.6	104	11	28.8	101	14
33	30.0	102	11	29.4	100	14
34	30.5	101	11	29.4	97	14
35	30.0	100	11	29.1	97	14
36	30.5	101	11	30.2	100	13
37	31.5	100	11	31.8	101	12
38	31.9	102	11	31.7	101	12
39	31.4	102	11	31.7	103	12
40	31.7	105	11	32.2	107	12
Mean for Weeks						
1-13	22.3	102		22.2	102	
14-40	28.8	103		28.8	102	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms of the skin. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Appendix B for male and female Tg.AC hemizygous mice.

Skin: The incidences of squamous cell papilloma of the vulva increased with a positive trend (P=0.018) (Tables 11 and B3). The incidences of squamous cell papilloma at all skin sites (including vulva) also increased with a positive trend ($P \leq 0.05$). Squamous cell papillomas of the vulva tended to be larger in treated mice (1 to 7 mm in greatest diameter; average diameter was 2.3 mm) than those in control mice (1 mm or less in greatest diameter). Size of papillomas did not seem to increase with increasing dose (data not shown).

TABLE 11
Incidences of Skin Neoplasms in Female Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Number Examined Microscopically	15	15	15	15	14	15
Vulva, Squamous Cell Papilloma, Multiple[a]	0	0	0	1	1	1
Vulva, Squamous Cell Papilloma (includes multiple)[b,c]	2	4	1	6	5	7
All Sites, Squamous Cell Papilloma[c,d]	4	6	3	7	8	9

[a] Number of animals with neoplasm
[b] Historical incidence for control Tg.AC female mice from 39- to 41-week studies (all routes): 3/99 (3%)
[c] Statistically significant ($P \leq 0.05$) by the Cochran-Armitage trend test (Armitage, 1971)
[d] Historical incidence for Tg.AC female mice: 43/99 (43%)

40-Week Gavage Study in C57BL/6 Mice

Survival

Estimates of 40-week survival probabilities for male and female mice are shown in Table 12. Survival of dosed mice was similar to that of the vehicle control groups, although three 8 mg/kg females died early.

Body Weights, Clinical Findings, and Organ Weights

Mean body weights were similar between dosed and vehicle control mice (Tables 13 and 14; Figure 4). There were no treatment-related clinical findings. There were no statistically significant differences in organ weights between the dosed and vehicle control groups (Table F5).

Pathology and Statistical Analyses

There were no chemical-related gross or microscopic findings in C57BL/6 mice administered allyl bromide for 40 weeks. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Appendix C for male and female C57BL/6 mice.

Table 12
Survival of C57BL/6 Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	8 mg/kg
Male		
Animals initially in study	15	15
Moribund	1	0
Animals surviving to study termination	14	15
Percent probability of survival at end of study[a]	93	100
Mean survival (days)[b]	279	280
Survival analysis[c]		P=1.000N
Female		
Animals initially in study	15	15
Moribund	0	2
Natural deaths	0	1
Animals surviving to study termination	15	12
Percent probability of survival at end of study	100	80
Mean survival (days)	279	273
Survival analysis		P=0.226

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group column. Lower mortality in a dosed group is indicated by **N**.

TABLE 13
Mean Body Weights and Survival of Male C57BL/6 Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	Vehicle Control		8 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.9	15	24.0	100	15
2	24.2	15	24.0	99	15
3	25.4	15	25.3	100	15
4	25.4	15	25.9	102	15
5	26.8	15	27.0	101	15
6	27.5	15	27.9	102	15
7	28.2	15	28.9	103	15
8	29.3	15	29.4	100	15
9	29.7	15	29.8	100	15
10	30.8	15	30.2	98	15
11	31.7	15	31.7	100	15
12	32.5	15	32.7	101	15
13	33.4	15	33.3	100	15
14	34.2	15	34.4	101	15
15	34.9	15	35.3	101	15
16	36.6	15	36.7	100	15
17	37.5	15	37.5	100	15
18	38.1	15	37.7	99	15
19	38.4	15	38.3	100	15
20	38.8	15	38.6	100	15
21	39.4	15	38.5	98	15
22	40.3	15	39.1	97	15
23	41.0	15	40.1	98	15
24	41.3	15	41.2	100	15
25	41.9	15	41.4	99	15
26	42.2	15	42.0	100	15
27	42.7	15	42.7	100	15
28	43.2	15	43.2	100	15
29	42.9	15	43.7	102	15
30	43.0	15	42.5	99	15
31	43.0	15	40.1	93	15
32	43.3	15	41.1	95	15
33	43.6	15	42.3	97	15
34	43.7	15	43.6	100	15
35	44.0	15	44.3	101	15
36	43.7	15	45.1	103	15
37	43.5	15	45.5	105	15
38	44.6	15	45.9	103	15
39	46.1	14	44.9	97	15
40	46.4	14	45.5	98	15
Mean for Weeks					
1-13	28.4		28.5	100	
14-40	41.4		41.2	99	

TABLE 14
Mean Body Weights and Survival of Female C57BL/6 Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	Vehicle Control		8 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	20.1	15	20.0	100	15
2	20.5	15	20.6	101	15
3	20.9	15	21.0	101	15
4	21.3	15	21.8	102	15
5	21.9	15	22.5	103	15
6	22.4	15	23.1	103	15
7	23.0	15	23.8	104	15
8	23.1	15	23.6	102	15
9	23.6	15	24.5	104	15
10	24.0	15	25.1	105	15
11	24.5	15	24.8	101	15
12	24.8	15	25.8	104	15
13	25.5	15	26.7	105	15
14	25.7	15	27.3	106	15
15	26.4	15	27.9	106	15
16	26.9	15	28.5	106	15
17	27.4	15	28.9	106	15
18	27.7	15	29.1	105	15
19	27.6	15	29.3	106	15
20	28.2	15	29.8	106	15
21	29.0	15	30.7	106	15
22	29.9	15	31.2	104	15
23	31.0	15	31.8	103	15
24	31.6	15	32.2	102	15
25	31.6	15	32.6	103	15
26	31.8	15	33.1	104	15
27	32.7	15	33.8	103	15
28	32.2	15	33.9	105	15
29	32.4	15	34.2	106	15
30	33.4	15	33.5	100	15
31	34.0	15	33.4	98	15
32	34.5	15	33.4	97	15
33	35.4	15	35.0	99	14
34	36.2	15	35.3	98	14
35	36.7	15	35.5	97	14
36	37.3	15	35.4	95	14
37	37.8	15	35.7	94	14
38	38.5	15	36.4	95	14
39	38.7	15	37.0	96	12
40	39.8	15	37.7	95	12
Mean for Weeks					
1-13	22.7		23.3	103	
14-40	32.4		32.7	101	

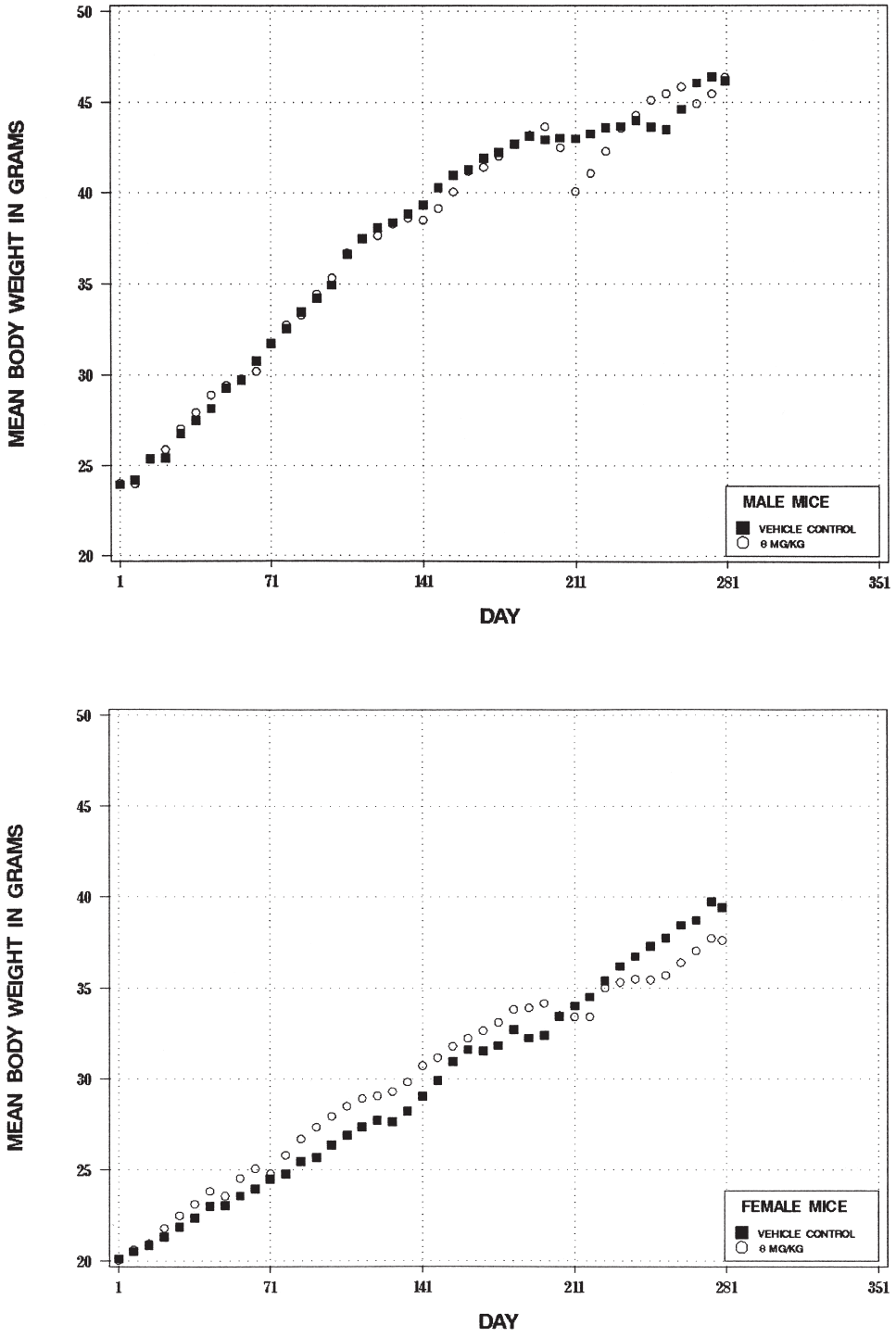


FIGURE 4
Growth Curves for Male and Female C57BL/6 Mice Exposed to Allyl Bromide by Gavage for 40 Weeks

40-Week Gavage Study in p53 Haploinsufficient Mice

Survival

Estimates of 40-week survival probabilities for male and female mice are shown in Table 15. Survival of dosed male and female mice was similar to that of the vehicle controls with no more than two deaths per group.

Body Weights, Clinical Findings, and Organ Weights

Mean body weights of 0.5, 4, and 8 mg/kg males were marginally greater than those of the vehicle controls after week 9 of the study (Figure 5 and Table 16). Mean body weights of 8 mg/kg females were marginally greater than those of the vehicle controls after week 10, and those of 4 mg/kg females were generally less after week 21 (Figure 5 and Table 17). There were no treatment-related clinical findings. Relative kidney and heart weights of females administered 4 mg/kg were significantly greater than those of the vehicle control group (Table F6).

Pathology and Statistical Analyses

There were no chemical-related gross or microscopic findings in p53 haploinsufficient mice administered allyl bromide for 40 weeks. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Appendix D for male and female p53 haploinsufficient mice.

Table 15
Survival of p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Male						
Animals initially in study	15	15	15	15	15	15
Moribund	0	0	0	0	1	0
Natural deaths	0	1	0	0	1	0
Animals surviving to study termination	15	14	15	15	13	15
Percent probability of survival at end of study[a]	100	93	100	100	87	100
Mean survival (days)[b]	274	272	274	274	271	274
Survival analysis[c]	P=1.000	P=1.000	—[d]	—	P=0.464	—
Female						
Animals initially in study	15	15	15	15	15	15
Moribund	2	1	2	0	0	1
Natural deaths	0	0	0	1	0	1
Animals surviving to study termination	13	14	13	14	15	13
Percent probability of survival at end of study	87	93	87	93	100	87
Mean survival (days)	272	275	271	275	277	276
Survival analysis	P=1.000N	P=0.984N	P=1.000	P=0.984N	P=0.464N	P=1.000N

[a] Kaplan-Meier determination
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dosed group is indicated by **N**.
[d] Value of statistic cannot be computed.

TABLE 16
Mean Body Weights and Survival of Male p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	Vehicle Control		0.5 mg/kg			1 mg/kg			2 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	24.6	15	25.2	102	15	24.3	99	15	24.6	100	15
2	25.0	15	25.0	100	15	24.3	97	15	24.9	100	15
3	25.6	15	25.7	100	15	25.6	100	15	25.8	101	15
4	26.4	15	27.2	103	15	25.7	97	15	26.3	100	15
5	27.2	15	28.6	105	15	26.9	99	15	27.6	102	15
6	27.8	15	29.0	104	15	27.7	100	15	28.0	101	15
7	29.0	15	29.8	103	15	28.6	99	15	29.3	101	15
8	29.8	15	31.3	105	15	29.6	99	15	30.4	102	15
9	30.6	15	32.3	106	15	30.8	101	15	30.6	100	15
10	31.5	15	33.6	107	15	31.6	100	15	31.9	101	15
11	32.3	15	34.6	107	15	32.4	100	15	33.2	103	15
12	33.0	15	35.3	107	15	33.6	102	15	34.2	104	15
13	34.0	15	36.0	106	15	34.7	102	15	35.2	104	15
14	34.7	15	36.9	106	15	35.7	103	15	35.9	104	15
15	35.8	15	38.1	106	15	36.5	102	15	36.9	103	15
16	36.7	15	39.0	106	15	37.9	103	15	37.5	102	15
17	37.3	15	39.9	107	15	38.7	104	15	38.3	103	15
18	37.9	15	41.1	108	15	39.4	104	15	39.4	104	15
19	38.5	15	41.6	108	15	39.8	103	15	39.8	103	15
20	39.2	15	42.2	108	15	40.2	103	15	40.2	103	15
21	39.7	15	42.8	108	15	41.1	104	15	40.7	103	15
22	40.6	15	43.4	107	15	41.8	103	15	41.3	102	15
23	41.1	15	44.2	108	15	42.8	104	15	42.1	102	15
24	41.5	15	44.7	108	15	43.3	104	15	42.6	103	15
25	41.7	15	45.2	108	15	43.6	105	15	42.9	103	15
26	42.4	15	45.4	107	15	44.3	105	15	43.3	102	15
27	42.9	15	45.5	106	15	44.6	104	15	43.6	102	15
28	43.4	15	45.7	105	15	44.9	104	15	44.2	102	15
29	43.9	15	46.6	106	15	44.7	102	15	45.0	103	15
30	43.5	15	46.6	107	15	44.8	103	15	45.3	104	15
31	44.1	15	46.7	106	15	45.4	103	15	45.5	103	15
32	44.0	15	47.0	107	15	46.1	105	15	46.0	105	15
33	44.7	15	48.0	107	15	46.9	105	15	46.3	104	15
34	44.7	15	48.4	108	15	46.8	105	15	45.9	103	15
35	45.2	15	49.2	109	14	47.8	106	15	46.8	104	15
36	45.6	15	49.2	108	14	47.2	104	15	46.4	102	15
37	45.8	15	49.9	109	14	47.9	105	15	45.0	98	15
38	46.7	15	50.1	107	14	48.6	104	15	46.2	99	15
39	46.8	15	50.0	107	14	49.2	105	15	47.8	102	15
40	47.5	15	49.4	104	14	49.9	105	15	48.7	103	15
Mean for Weeks											
1-13	29.0		30.3	104		28.9	100		29.4	101	
14-40	42.1		45.1	107		43.7	104		43.1	103	

TABLE 16
Mean Body Weights and Survival of Male p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	4 mg/kg Av. Wt. (g)	4 mg/kg Wt. (% of controls)	4 mg/kg No. of Survivors	8 mg/kg Av. Wt. (g)	8 mg/kg Wt. (% of controls)	8 mg/kg No. of Survivors
1	24.9	101	15	25.1	102	15
2	25.1	100	15	25.2	101	15
3	26.1	102	15	26.1	102	15
4	27.1	103	15	27.0	102	15
5	28.4	104	15	28.1	103	15
6	28.8	104	15	28.9	104	15
7	29.2	101	15	30.0	103	15
8	30.7	103	15	31.2	105	15
9	32.2	105	15	32.1	105	15
10	33.6	107	15	33.2	105	15
11	34.6	107	15	33.9	105	15
12	35.5	108	15	34.9	106	15
13	36.5	107	15	35.6	105	15
14	37.3	108	15	36.6	106	15
15	38.2	107	15	37.9	106	15
16	39.3	107	15	38.9	106	15
17	39.8	107	15	39.8	107	15
18	39.9	105	15	40.6	107	15
19	39.7	103	15	41.2	107	15
20	40.1	102	15	41.6	106	15
21	41.7	105	15	42.0	106	15
22	42.2	104	15	42.8	105	15
23	43.2	105	15	43.4	106	15
24	43.9	106	15	44.0	106	15
25	44.0	106	15	44.5	107	15
26	44.7	105	15	45.1	106	15
27	44.9	105	15	45.7	107	15
28	45.5	105	15	46.0	106	15
29	45.9	105	15	46.0	105	15
30	46.4	107	15	46.4	107	15
31	46.7	106	15	46.9	106	15
32	46.0	105	15	47.6	108	15
33	47.8	107	14	48.2	108	15
34	46.6	104	14	48.1	108	15
35	46.8	104	14	49.3	109	15
36	47.1	103	14	49.4	108	15
37	47.9	105	14	49.2	107	15
38	48.6	104	14	49.5	106	15
39	49.3	105	14	50.2	107	15
40	49.8	105	13	50.5	106	15
Mean for Weeks						
1-13	30.2	104		30.1	104	
14-40	44.2	105		44.9	107	

TABLE 17
Mean Body Weights and Survival of Female p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	Vehicle Control		0.5 mg/kg			1 mg/kg			2 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	20.0	15	20.1	101	15	19.6	98	15	20.1	101	15
2	20.2	15	20.2	100	15	19.6	97	15	20.2	100	15
3	21.0	15	21.0	100	15	20.6	98	15	20.8	99	15
4	22.1	15	21.9	99	15	21.1	96	15	21.4	97	15
5	22.4	15	22.5	100	15	22.1	99	15	22.1	99	15
6	22.6	15	22.7	100	15	22.3	99	15	22.3	99	15
7	23.2	15	22.9	99	15	22.9	99	15	22.7	98	15
8	23.6	15	23.7	100	15	23.4	99	15	22.8	97	15
9	24.0	15	23.9	100	15	23.9	100	15	23.9	100	15
10	24.6	15	24.6	100	15	24.5	100	15	24.3	99	15
11	24.6	15	25.6	104	15	25.0	102	15	24.8	101	15
12	25.3	15	25.8	102	15	25.8	102	15	25.3	100	15
13	25.7	15	26.5	103	15	26.5	103	15	25.8	100	15
14	26.2	15	26.7	102	15	26.5	101	15	26.6	102	15
15	27.3	15	28.0	103	15	27.5	101	15	27.4	100	15
16	27.8	15	28.2	101	15	28.8	104	15	28.1	101	15
17	28.7	15	29.1	101	15	29.4	102	15	28.7	100	15
18	29.0	15	30.0	103	15	30.0	103	15	29.3	101	15
19	29.7	15	31.0	104	15	29.8	100	15	29.5	99	15
20	30.7	15	31.8	104	15	30.3	99	15	30.2	98	15
21	31.3	15	32.5	104	15	31.3	100	15	31.0	99	15
22	32.6	15	33.2	102	15	32.0	98	15	31.6	97	15
23	33.4	15	33.8	101	15	33.1	99	15	32.8	98	15
24	33.8	15	34.5	102	15	33.8	100	15	33.1	98	15
25	34.1	15	34.8	102	15	34.3	101	15	33.3	98	15
26	34.4	15	35.7	104	15	34.8	101	15	34.1	99	15
27	35.3	15	36.2	103	15	35.3	100	15	34.5	98	15
28	36.2	15	37.3	103	15	35.9	99	15	35.0	97	15
29	36.7	15	38.3	104	15	36.2	99	15	35.8	98	15
30	36.8	15	38.1	104	15	36.1	98	15	36.4	99	15
31	36.4	15	38.6	106	15	36.2	100	15	36.7	101	15
32	36.4	15	39.5	109	15	36.6	101	15	37.1	102	15
33	37.6	14	40.1	107	15	38.2	102	14	37.0	98	15
34	37.9	14	39.8	105	15	38.1	101	14	37.4	99	15
35	38.3	14	40.1	105	15	39.9	104	13	37.8	99	15
36	39.6	14	40.5	102	15	41.0	104	13	38.3	97	15
37	40.2	14	42.5	106	14	41.7	104	13	39.0	97	14
38	42.5	13	43.3	102	14	42.6	100	13	40.2	95	14
39	42.7	13	43.4	102	14	42.7	100	13	41.5	97	14
40	43.0	13	43.4	101	14	43.8	102	13	40.3	94	14
Mean for Weeks											
1-13	23.0		23.2	101		22.9	99		22.8	99	
14-40	34.8		35.9	103		35.0	101		34.2	99	

TABLE 17
Mean Body Weights and Survival of Female p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

Weeks on Study	4 mg/kg Av. Wt. (g)	4 mg/kg Wt. (% of controls)	4 mg/kg No. of Survivors	8 mg/kg Av. Wt. (g)	8 mg/kg Wt. (% of controls)	8 mg/kg No. of Survivors
1	20.1	101	15	19.9	100	15
2	20.3	101	15	20.3	101	15
3	21.0	100	15	20.8	99	15
4	21.6	98	15	21.3	96	15
5	22.3	100	15	22.3	100	15
6	22.8	101	15	22.9	101	15
7	23.0	99	15	23.1	100	15
8	23.3	99	15	23.7	100	15
9	24.0	100	15	24.6	103	15
10	23.9	97	15	25.1	102	15
11	24.7	100	15	25.9	105	15
12	25.1	99	15	26.7	106	15
13	25.6	100	15	27.9	109	15
14	26.1	100	15	28.4	108	15
15	27.0	99	15	29.7	109	15
16	27.3	98	15	30.5	110	15
17	27.5	96	15	31.4	109	15
18	27.9	96	15	32.0	110	15
19	28.2	95	15	32.4	109	15
20	29.1	95	15	33.6	109	15
21	29.8	95	15	34.5	110	15
22	30.0	92	15	35.4	109	15
23	30.7	92	15	36.3	109	15
24	31.1	92	15	37.2	110	15
25	31.1	91	15	37.6	110	15
26	31.7	92	15	38.1	111	15
27	32.3	92	15	39.1	111	15
28	32.9	91	15	39.7	110	15
29	33.9	92	15	40.4	110	15
30	34.0	92	15	41.0	111	15
31	33.9	93	15	41.1	113	15
32	34.6	95	15	42.0	115	15
33	35.1	93	15	42.2	112	15
34	35.4	93	15	41.8	110	15
35	36.2	95	15	42.0	110	15
36	36.3	92	15	42.0	106	15
37	36.6	91	15	41.1	102	15
38	37.1	87	15	41.8	98	15
39	37.7	88	15	42.4	99	15
40	37.7	88	15	45.2	105	13
Mean for Weeks						
1-13	22.9	100		23.4	102	
14-40	32.3	93		37.7	109	

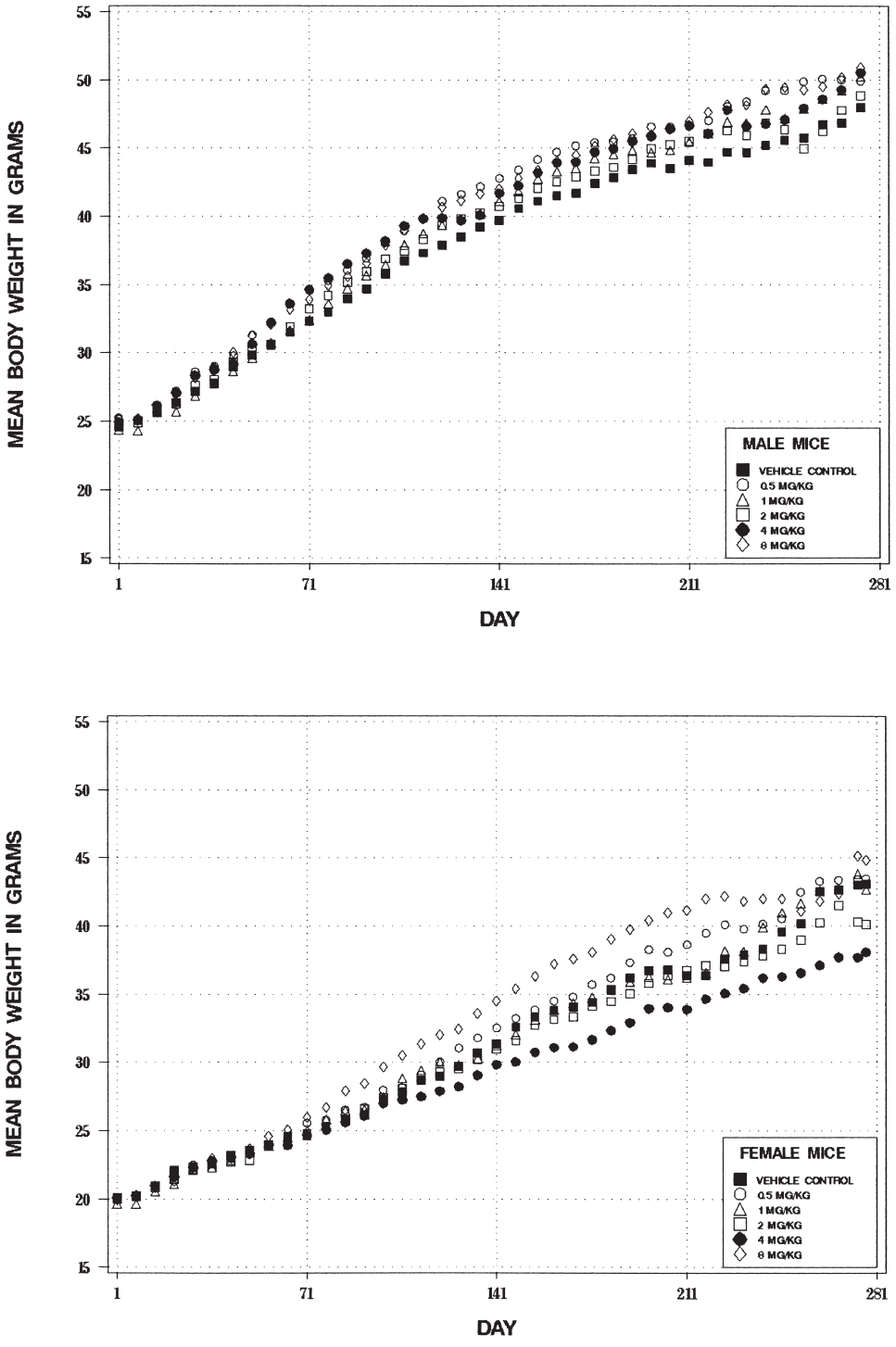


FIGURE 5
Growth Curves for Male and Female p53 Haploinsufficient Mice Exposed to Allyl Bromide by Gavage for 40 Weeks

GENETIC TOXICOLOGY

Allyl bromide was mutagenic in *Salmonella typhimurium* strain TA100, with and without Aroclor-induced rat or hamster liver S9 (Table E1). The concentration ranges tested were 10 to 1,000 µg/plate without S9 and 3 to 333 µg/plate with S9; significant increases in revertants occurred at concentrations of 100 µg/plate and above. The mutagenic response obtained in the absence of S9 was stronger than that observed with either rat or hamster liver S9. No mutagenicity was detected in the *S. typhimurium* strain TA98, with or without S9, over the same concentration ranges.

The frequency of micronucleated erythrocytes was assessed in each of the four mouse strains treated with allyl bromide for 40 weeks. Results in all four strains of mice were concluded to be negative; in addition, no significant, consistent changes in the percentage of polychromatic erythrocytes (reticulocytes) among total erythrocytes were observed in any of the four strains (Tables E2, E3, E4, and E5).

Some observations of note in these micronucleus tests include the small increase in micronucleated erythrocytes seen in the single dosed group (8 mg/kg) of female C57BL/6 mice that was evaluated for micronucleus frequency (Table E4). Although the P value was significant ($\leq$0.05), these results were judged to be negative because the small increase represented less than half a micronucleus per 1,000 cells, which is not biologically relevant. In the male p53 haploinsufficient mice, one dosed group (1.0 mg/kg) showed a small but significant increase (P=0.0006) in micronucleated erythrocyte frequency, but none of the three higher doses showed an effect; therefore, this small increase at a single dose concentration in one sex, even though statistically significant ($P \leq 0.005$), was not considered sufficient evidence of the ability of allyl bromide to induce an effect in this assay.

DISCUSSION AND CONCLUSIONS

These studies were performed to determine the toxicity and carcinogenicity of allyl bromide in Tg.AC hemizygous and p53 haploinsufficient mice. No conventional 2-year bioassays have been conducted on allyl bromide.

The 2-week studies were conducted in the parent strains of the Tg.AC hemizygous and p53 haploinsufficient mice, which were FVB/N and C57BL/6 mice, respectively. In the 2-week dermal studies in FVB/N mice, there were no treatment-related histopathologic changes or treatment-related effects on body weight or mortality. The volatility of allyl bromide may have resulted in limited exposure by dermal administration.

In the 2-week gavage study conducted in C57BL/6 mice, there were biologically significant increases in the incidences of forestomach lesions at 15, 30, 60, and 120 mg/kg. In 14-week gavage studies on the allyl bromide metabolites allyl alcohol and acrolein, the forestomach was also the target organ for toxicity in F344/N rats and $B6C3F_1$ mice (NTP, 2006). Allyl alcohol caused forestomach hyperplasia in mice (but not in rats) at 12, 25, and 50 mg/kg. Acrolein caused forestomach hyperplasia in rats at 10 mg/kg and forestomach or glandular stomach toxicity and/or necrosis in mice at 5, 10, and 20 mg/kg. Thus, the forestomach was a target organ for toxicity after administration of allyl bromide or its metabolites, allyl alcohol or acrolein.

Because of the lack of toxicity via the dermal route of exposure in the 2-week allyl bromide study, the gavage route of administration was chosen for the 40-week studies in male and female FVB/N mice, Tg.AC hemizygous mice, C57BL/6 mice, and p53 haploinsufficient mice using a high dose of 8 mg/kg.

Activation of the *ras* gene in Tg.AC mice is under the control of the zeta-globin promoter. The v-Ha-*ras* structural gene has a terminal simian virus 40 polyadenylation signal (Thompson *et al.*, 1998). In some previous studies with Tg.AC mice, a nonresponsive phenotype was identified, and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) failed to induce skin papillomas (Thompson *et al.*, 1998, 2001). Therefore, TPA is included as a positive control in Tg.AC mouse studies to test whether the mice have the full spectrum of genetic components of the v-Ha-*ras* gene for skin tumor induction. In this Tg.AC allyl bromide study, TPA did induce skin papillomas, and the Tg.AC mice used were considered to contain the necessary promoters and v-Ha-*ras* gene components.

In the 40-week study conducted in Tg.AC mice, the increase in total skin papillomas and vulvar tumors in female mice was significant by the Cochran-Armitage trend statistic. One hypothesis for this effect is the formation of a reactive metabolite derived from allyl mercapturic acid sulfoxide that was excreted in the urine, was absorbed by surrounding tissue (such as vulvar cells), and caused DNA damage (Kaye *et al.*, 1972; Schuphan and Casida, 1979a,b). There were no treatment-related lesions in the forestomach.

In humans, there are two pathways leading to vulvar tumors: one pathway is associated with exposure to human papilloma virus (HPV), the other is an HPV-independent pathway (van der Avoort *et al.*, 2005). The hypothesis that an HPV-independent pathway may lead to vulvar tumors is supported by the current study and by treatment-related vulvar squamous cell papillomas seen in a cyclophosphamide gavage study conducted in Tg.AC mice (Eastin *et al.*, 2001). Cyclophosphamide and metabolites are excreted in urine, which may expose vulva cells to carcinogens (Sottani *et al.*, 2005). Allyl bromide and cyclophosphamide (Balu *et al.*, 2002) are both metabolized to acrolein and likely have other metabolites in common. It remains to be demonstrated if the common metabolites are responsible for the common response.

There were no significant allyl bromide treatment-related increases in tumors in the other mice studied (FVB/N, C57BL/6, and p53 haploinsufficient mice). Studies of the allyl bromide metabolite acrolein also showed no evidence for a carcinogenic effect in rats

or mice. A study on the potential of acrolein to cause cancer in CD-1 mice receiving 0, 0.5, 2, or 4.5 mg/kg per day by gavage for 18 months showed no evidence, nor did an acrolein study in Sprague-Dawley rats receiving 0, 0.05, 0.5, or 2.5 mg/kg per day by gavage for 18 months (Parent *et al.*, 1991, 1992). No acrolein-induced cancers were seen when the chemical was administered in the drinking water to F344/N rats for up to 2 years (Linjinsky and Reuber, 1987; Linjinsky, 1988).

In NTP studies, allyl bromide was mutagenic in *S. typhimurium* strain TA100, with and without liver activation enzymes. The frequencies of micronucleated erythrocytes were determined at terminal sacrifice in the transgenic strains and their corresponding parent strains; no treatment-related increases in micronucleated erythrocytes were observed in male or female mice in any of the four strains. The erythrocyte micronucleus assay detects numerical or structural chromosomal damage induced in nucleated precursor cells in the bone marrow. Allyl bromide, a direct alkylating agent, may bind to proteins in blood, which might prevent the chemical or mutagenic metabolites from reaching the bone marrow. Alternatively, allyl bromide may induce point mutations exclusively.

CONCLUSIONS

Under the conditions of this study, there was *no evidence of carcinogenic activity** in male or female p53 haploinsufficient mice administered allyl bromide at 0.5, 1, 2, 4, or 8 mg/kg per day by corn oil gavage, 5 days a week for 40 weeks.

There was a marginal increase in the incidence of squamous cell papillomas, primarily of the vulva, in female Tg.AC hemizygous mice administered allyl bromide by corn oil gavage for 40 weeks. No treatment-related neoplasms were seen in male Tg.AC hemizygous mice administered allyl bromide by gavage at 0.5, 1, 2, 4, or 8 mg/kg, 5 days per week for 40 weeks.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Report appears on page 11.

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APPENDIX A
SUMMARY OF LESIONS IN FVB/N MICE IN THE 40-WEEK GAVAGE STUDY OF ALLYL BROMIDE

TABLE A1
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Male FVB/N Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	8 mg/kg
Disposition Summary		
Animals initially in study	15	15
Early death		
Accidental death		1
Survivors		
Terminal sacrifice	15	14
Animals examined microscopically	15	15
Alimentary System		
Esophagus		(1)
Epithelium, hyperplasia, diffuse		1 (100%)
Muscularis, periesophageal tissue, inflammation, chronic active, focal		1 (100%)
Liver	(15)	(15)
Tension lipidosis		1 (7%)
Hepatocyte, necrosis, focal	1 (7%)	1 (7%)
Hepatocyte, vacuolization cytoplasmic, focal	1 (7%)	
Hepatocyte, centrilobular, vacuolization cytoplasmic	3 (20%)	2 (13%)
Hepatocyte, centrilobular, vacuolization cytoplasmic, focal	1 (7%)	
Serosa, mineralization, focal	1 (7%)	
Cardiovascular System		
None		
Endocrine System		
Adrenal cortex	(15)	(14)
Atrophy	15 (100%)	14 (100%)
Hypertrophy, focal	13 (87%)	14 (100%)
Subcapsular, hyperplasia, focal	1 (7%)	
Thyroid gland	(15)	(15)
Ectopic thymus	1 (7%)	
General Body System		
None		
Genital System		
Preputial gland	(2)	(2)
Ectasia	2 (100%)	2 (100%)
Infiltration cellular, focal, lymphocyte		1 (50%)
Testes	(15)	(15)
Germinal epithelium, degeneration, focal	2 (13%)	2 (13%)
Hematopoietic System		
Lymph node, mediastinal	(14)	(15)
Hyperplasia	1 (7%)	
Spleen	(15)	(15)
Hematopoietic cell proliferation	15 (100%)	13 (87%)
Pigmentation	11 (73%)	11 (73%)
Lymphoid follicle, atrophy		1 (7%)

TABLE A1
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Male FVB/N Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	8 mg/kg
Hematopoietic System (continued)		
Thymus	(15)	(15)
Atrophy, diffuse		1 (7%)
Atrophy, focal	2 (13%)	3 (20%)
Hyperplasia, focal		1 (7%)
Integumentary System		
Skin	(15)	(15)
Subcutaneous tissue, inflammation, chronic active, focal	1 (7%)	1 (7%)
Musculoskeletal System		
None		
Nervous System		
None		
Respiratory System		
Lung	(15)	(15)
Alveolar/bronchiolar adenoma		2 (13%)
Alveolar/bronchiolar adenoma, multiple		1 (7%)
Hemorrhage, focal		1 (7%)
Alveolar epithelium, hyperplasia, focal	1 (7%)	1 (7%)
Mediastinum, inflammation, acute, focal		1 (7%)
Perivascular, infiltration cellular, focal lymphocyte		1 (7%)
Special Senses System		
None		
Urinary System		
Kidney	(15)	(15)
Renal tubule, degeneration, focal		1 (7%)
Renal tubule, dilatation, diffuse	1 (7%)	
Renal tubule, dilatation, focal	1 (7%)	
Neoplasm Summary		
Total animals with primary neoplasms[b]		3
Total primary neoplasms		3
Total animals with benign neoplasms		3
Total benign neoplasms		3

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female FVB/N Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	8 mg/kg
Disposition Summary		
Animals initially in study	15	15
Early death		
Moribund		1
Survivors		
Terminal sacrifice	15	14
Animals examined microscopically	15	15
Alimentary System		
Liver	(15)	(15)
Hepatocyte, necrosis, focal	2 (13%)	2 (13%)
Hepatocyte, vacuolization cytoplasmic, diffuse	2 (13%)	2 (13%)
Hepatocyte, vacuolization cytoplasmic, focal	2 (13%)	3 (20%)
Hepatocyte, centrilobular, vacuolization cytoplasmic		1 (7%)
Kupffer cell, hyperplasia, diffuse		1 (7%)
Cardiovascular System		
None		
Endocrine System		
Adrenal cortex	(15)	(15)
Atrophy	2 (13%)	2 (13%)
Subcapsular, hyperplasia, focal	8 (53%)	9 (60%)
Zona reticularis, vacuolization cytoplasmic, diffuse	12 (80%)	13 (87%)
Zona reticularis, vacuolization cytoplasmic, focal	2 (13%)	2 (13%)
General Body System		
None		
Genital System		
Ovary	(15)	(15)
Hemorrhage, focal		2 (13%)
Inflammation, acute, focal		1 (7%)
Inflammation, chronic active, focal		1 (7%)
Uterus	(15)	(15)
Hydrometra	2 (13%)	1 (7%)
Endometrium, hyperplasia, cystic	14 (93%)	15 (100%)
Hematopoietic System		
Spleen	(15)	(15)
Hematopoietic cell proliferation	15 (100%)	15 (100%)
Pigmentation	15 (100%)	14 (93%)
Lymphoid follicle, hyperplasia	1 (7%)	
Thymus	(15)	(14)
Atrophy, focal	4 (27%)	3 (21%)

TABLE A2
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female FVB/N Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	8 mg/kg
Integumentary System		
None		
Musculoskeletal System		
None		
Nervous System		
None		
Respiratory System		
Lung	(15)	(15)
Alveolar/bronchiolar adenoma	2 (13%)	1 (7%)
Alveolar epithelium, hyperplasia, focal	1 (7%)	
Alveolus, hemorrhage, focal	1 (7%)	
Special Senses System		
None		
Urinary System		
Kidney	(15)	(15)
Renal tubule, dilatation, focal	2 (13%)	2 (13%)
Neoplasm Summary		
Total animals with primary neoplasms[b]	2	1
Total primary neoplasms	2	1
Total animals with benign neoplasms	2	1
Total benign neoplasms	2	1

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

APPENDIX B
SUMMARY OF LESIONS IN TG.AC HEMIZYGOUS MICE IN THE 40-WEEK GAVAGE STUDY OF ALLYL BROMIDE

TABLE B1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Accidental deaths		1		1	1	1
Moribund	2	2	3	2	4	1
Natural deaths	1	3	3		4	2
Survivors						
Terminal sacrifice	12	9	9	12	6	11
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(14)	(15)	(14)	(15)	(14)	(15)
Leukemia erythrocytic			1 (7%)		1 (7%)	1 (7%)
Salivary glands		(1)	(1)	(1)		
Duct, carcinoma		1 (100%)	1 (100%)	1 (100%)		
Stomach, forestomach	(15)	(14)	(14)	(15)	(13)	(14)
Squamous cell papilloma	5 (33%)	3 (21%)	5 (36%)	5 (33%)	5 (38%)	5 (36%)
Squamous cell papilloma, multiple	1 (7%)	2 (14%)	1 (7%)	1 (7%)		
Tooth	(5)	(5)	(4)	(3)	(6)	(4)
Odontogenic tumor	5 (100%)	5 (100%)	3 (75%)	3 (100%)	6 (100%)	3 (75%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Spleen	(15)	(14)	(14)	(15)	(14)	(15)
Leukemia erythrocytic			1 (7%)		1 (7%)	1 (7%)
Integumentary System						
Skin	(15)	(15)	(15)	(15)	(14)	(15)
Keratoacanthoma			3 (20%)			
Squamous cell papilloma	4 (27%)	1 (7%)	5 (33%)	2 (13%)	1 (7%)	1 (7%)
Squamous cell papilloma, multiple	1 (7%)		2 (13%)	1 (7%)	1 (7%)	3 (20%)
Conjunctiva, squamous cell carcinoma					1 (7%)	
Lip, squamous cell papilloma		1 (7%)	1 (7%)			1 (7%)
Lip, squamous cell papilloma, multiple	1 (7%)			1 (7%)		

TABLE B1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(15)	(15)	(14)	(15)	(14)	(15)
Alveolar/bronchiolar adenoma	1 (7%)			1 (7%)	2 (14%)	
Alveolar/bronchiolar carcinoma		1 (7%)				
Carcinoma, metastatic, salivary glands			1 (7%)			
Leukemia erythrocytic			1 (7%)			
Special Senses System						
None						
Urinary System						
None						
Systemic Lesions						
Multiple organs[b]	(15)	(15)	(15)	(15)	(15)	(15)
Leukemia erythrocytic			1 (7%)		1 (7%)	1 (7%)
Neoplasm Summary						
Total animals with primary neoplasms[c]	11	10	11	11	12	10
Total primary neoplasms	18	14	22	15	17	14
Total animals with benign neoplasms	9	5	9	9	8	7
Total benign neoplasms	13	7	17	11	9	10
Total animals with malignant neoplasms		2	2	1	2	1
Total malignant neoplasms		2	2	1	2	1
Total animals with metastatic neoplasms			1			
Total metastatic neoplasms			1			
Total animals with uncertain neoplasms-benign or malignant	5	5	3	3	6	3
Total uncertain neoplasms	5	5	3	3	6	3

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Accidental deaths		1		1	1	1
Moribund	2	2	3	2	4	1
Natural deaths	1	3	3		4	2
Survivors						
Terminal sacrifice	12	9	9	12	6	11
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Esophagus		(1)		(1)	(1)	(1)
Muscularis, periesophageal tissue, inflammation, chronic active, diffuse				1 (100%)		
Periesophageal tissue, inflammation, chronic active, diffuse					1 (100%)	1 (100%)
Periesophageal tissue, inflammation, chronic active, focal		1 (100%)				
Liver	(14)	(15)	(14)	(15)	(14)	(15)
Necrosis, focal		1 (7%)				1 (7%)
Hepatocyte, necrosis, focal	2 (14%)	3 (20%)	5 (36%)		2 (14%)	
Hepatocyte, vacuolization cytoplasmic, diffuse		1 (7%)				
Hepatocyte, vacuolization cytoplasmic, focal				1 (7%)	1 (7%)	2 (13%)
Hepatocyte, centrilobular, hypertrophy	1 (7%)					
Hepatocyte, centrilobular, vacuolization cytoplasmic	2 (14%)		2 (14%)			1 (7%)
Serosa, inflammation, chronic active, focal		1 (7%)				
Sinusoid, inflammation, focal			1 (7%)			
Mesentery	(1)	(1)		(2)		
Fat, necrosis, focal	1 (100%)	1 (100%)		2 (100%)		
Pancreas						(1)
Acinus, atrophy, diffuse						1 (100%)
Salivary glands		(1)	(1)	(1)		
Necrosis, focal			1 (100%)			
Tooth	(5)	(5)	(4)	(3)	(6)	(4)
Peridontal tissue, inflammation, chronic active, focal						1 (25%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(15)	(14)	(14)	(14)	(12)	(14)
Atrophy	15 (100%)	13 (93%)	14 (100%)	14 (100%)	10 (83%)	12 (86%)
Degeneration, focal			1 (7%)			
Hypertrophy, focal	13 (87%)	10 (71%)	9 (64%)	11 (79%)	10 (83%)	11 (79%)
Subcapsular, hyperplasia, focal				1 (7%)		

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

TABLE B2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Endocrine System (continued)						
Adrenal cortex (continued)	(15)	(14)	(14)	(14)	(12)	(14)
Zona reticularis, vacuolization cytoplasmic, focal	1 (7%)			1 (7%)	1 (8%)	1 (7%)
Thyroid gland	(15)	(13)	(14)	(15)	(9)	(14)
Inflammation, chronic active, focal		1 (8%)				
General Body System						
Tissue NOS			(1)			(1)
Fat, necrosis, focal						1 (100%)
Mediastinum, inflammation, chronic active focal			1 (100%)			
Genital System						
Preputial gland	(1)	(2)	(2)	(1)	(4)	(2)
Ectasia	1 (100%)	1 (50%)	2 (100%)	1 (100%)	4 (100%)	2 (100%)
Inflammation, chronic active, focal		1 (50%)				
Seminal vesicle				(1)		(1)
Dilatation				1 (100%)		
Bilateral, dilatation						1 (100%)
Testes	(15)	(15)	(15)	(15)	(14)	(14)
Germinal epithelium, degeneration, diffuse				1 (7%)		
Germinal epithelium, degeneration, focal	1 (7%)		5 (33%)	1 (7%)		
Hematopoietic System						
Lymph node, mandibular	(15)	(13)	(14)	(15)	(13)	(15)
Hyperplasia	1 (7%)	1 (8%)	2 (14%)		3 (23%)	1 (7%)
Hyperplasia, histiocytic		1 (8%)				
Lymph node, mesenteric	(14)	(14)	(14)	(15)	(12)	(14)
Hyperplasia		1 (7%)				
Inflammation, chronic active, focal			1 (7%)			
Lymph node, mediastinal	(14)	(13)	(14)	(15)	(10)	(14)
Hyperplasia		1 (8%)	1 (7%)			
Spleen	(15)	(14)	(14)	(15)	(14)	(15)
Congestion		1 (7%)			1 (7%)	
Hematopoietic cell proliferation	14 (93%)	14 (100%)	13 (93%)	15 (100%)	12 (86%)	13 (87%)
Inflammation, chronic active, focal			1 (7%)			
Pigmentation	8 (53%)	10 (71%)	9 (64%)	12 (80%)	6 (43%)	11 (73%)
Lymphoid follicle, depletion cellular	1 (7%)	1 (7%)		1 (7%)	1 (7%)	
Thymus	(15)	(13)	(12)	(15)	(10)	(13)
Atrophy, diffuse	3 (20%)	3 (23%)	2 (17%)	2 (13%)	3 (30%)	1 (8%)
Atrophy, focal	1 (7%)	2 (15%)	1 (8%)	4 (27%)	1 (10%)	1 (8%)
Hyperplasia			2 (17%)			
Hyperplasia, focal					1 (10%)	
Integumentary System						
Skin	(15)	(15)	(15)	(15)	(14)	(15)
Hyperkeratosis, focal						1 (7%)
Dermis, inflammation, chronic active, focal						1 (7%)
Epidermis, hyperplasia, focal	1 (7%)					2 (13%)

TABLE B2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Integumentary System (continued)						
Skin (continued)	(15)	(15)	(15)	(15)	(14)	(15)
Subcutaneous tissue, inflammation, chronic active, focal		1 (7%)		1 (7%)		
Subcutaneous tissue, necrosis, focal			1 (7%)			1 (7%)
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(15)	(15)	(14)	(15)	(14)	(15)
Inflammation, chronic active, focal			1 (7%)			
Alveolar epithelium, hyperplasia, focal		1 (7%)				
Alveolus, hemorrhage, focal			1 (7%)	1 (7%)		1 (7%)
Arteriole, artery, inflammation, chronic active, focal					2 (14%)	
Arteriole, capillary, perivascular, inflammation, acute		1 (7%)				
Mediastinum, inflammation, chronic active, diffuse						1 (7%)
Perivascular, infiltration cellular, focal, lymphocyte		1 (7%)	1 (7%)			
Perivascular, infiltration cellular, lymphocyte	1 (7%)				1 (7%)	1 (7%)
Serosa, mediastinum, inflammation, chronic active, diffuse			1 (7%)			
Special Senses System						
Eye	(1)				(1)	
Retina, atrophy	1 (100%)				1 (100%)	
Urinary System						
Kidney	(15)	(14)	(14)	(15)	(14)	(14)
Inflammation, acute, focal		1 (7%)				
Inflammation, chronic active, focal			1 (7%)			
Bilateral, cortex, inflammation, acute, focal					1 (7%)	
Capsule, cortex, inflammation, chronic active, focal		1 (7%)				
Cortex, inflammation, acute, focal					2 (14%)	
Renal tubule, degeneration, focal					2 (14%)	
Renal tubule, dilatation, diffuse			1 (7%)		1 (7%)	
Renal tubule, dilatation, focal	1 (7%)	1 (7%)	1 (7%)	1 (7%)		

TABLE B3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Accidental deaths	2					
Moribund	2	3	4	6	1	3
Natural deaths	2	2	3	1	3	
Survivors						
Terminal sacrifice	9	10	8	8	11	12
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(14)	(15)
Leukemia erythrocytic		1 (7%)				
Lymphoma malignant	2 (13%)	1 (7%)		1 (7%)		
Salivary glands			(3)			
Duct, carcinoma			3 (100%)			
Stomach, forestomach	(14)	(15)	(12)	(13)	(14)	(15)
Squamous cell papilloma	5 (36%)	8 (53%)	4 (33%)	7 (54%)	5 (36%)	6 (40%)
Squamous cell papilloma, multiple	3 (21%)	2 (13%)	2 (17%)		2 (14%)	4 (27%)
Tooth	(2)	(3)	(6)	(7)	(3)	(3)
Odontogenic tumor	2 (100%)	3 (100%)	6 (100%)	6 (86%)	2 (67%)	3 (100%)
Cardiovascular System						
Heart		(1)		(1)		
Lymphoma malignant		1 (100%)				
Endocrine System						
Adrenal cortex	(13)	(14)	(12)	(13)	(12)	(15)
Lymphoma malignant		1 (7%)				
Adrenal medulla	(13)	(14)	(12)	(12)	(12)	(15)
Lymphoma malignant		1 (7%)				
Pituitary gland	(11)	(14)	(14)	(13)	(14)	(14)
Lymphoma malignant				1 (8%)		
General Body System						
None						
Genital System						
Ovary	(13)	(15)	(14)	(14)	(13)	(15)
Lymphoma malignant	1 (8%)	1 (7%)				
Uterus	(13)	(15)	(13)	(14)	(12)	(15)
Lymphoma malignant		1 (7%)				

TABLE B3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Hematopoietic System						
Lymph node, mesenteric	(13)	(14)	(12)	(15)	(12)	(15)
Lymphoma malignant		1 (7%)				
Lymph node, mediastinal	(14)	(15)	(14)	(14)	(12)	(14)
Lymphoma malignant		1 (7%)				
Spleen	(15)	(14)	(15)	(15)	(13)	(15)
Leukemia erythrocytic		1 (7%)				
Lymphoma malignant	2 (13%)	1 (7%)		1 (7%)		
Integumentary System						
Skin	(15)	(15)	(15)	(15)	(14)	(15)
Squamous cell papilloma	1 (7%)	1 (7%)	3 (20%)	3 (20%)	5 (36%)	1 (7%)
Squamous cell papilloma, multiple					1 (7%)	
Dermis, fibrosarcoma						1 (7%)
Lip, squamous cell papilloma	1 (7%)	1 (7%)			1 (7%)	3 (20%)
Vulva, squamous cell papilloma	2 (13%)	4 (27%)	1 (7%)	5 (33%)	4 (29%)	6 (40%)
Vulva, squamous cell papilloma, multiple				1 (7%)	1 (7%)	1 (7%)
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(14)	(15)	(15)	(15)	(14)	(15)
Alveolar/bronchiolar adenoma				2 (13%)		
Alveolar/bronchiolar carcinoma						1 (7%)
Lymphoma malignant		1 (7%)		1 (7%)		
Special Senses System						
Harderian gland						(1)
Adenoma						1 (100%)
Urinary System						
Kidney	(15)	(14)	(14)	(15)	(13)	(15)
Lymphoma malignant	1 (7%)	1 (7%)		1 (7%)		

TABLE B3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Systemic Lesions						
Multiple organs[b]	(15)	(15)	(15)	(15)	(15)	(15)
Leukemia erythrocytic		1 (7%)				
Lymphoma malignant	2 (13%)	1 (7%)		1 (7%)		
Neoplasm Summary						
Total animals with primary neoplasms[c]	10	13	10	14	11	14
Total primary neoplasms	16	21	19	25	21	27
Total animals with benign neoplasms	9	10	8	9	11	14
Total benign neoplasms	12	16	10	18	19	22
Total animals with malignant neoplasms	2	2	3	1		2
Total malignant neoplasms	2	2	3	1		2
Total animals with uncertain neoplasms-benign or malignant	2	3	6	6	2	3
Total uncertain neoplasms	2	3	6	6	2	3

[a] Number of animals examined microscopically at site and number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Accidental deaths	2					
Moribund	2	3	4	6	1	3
Natural deaths	2	2	3	1	3	
Survivors						
Terminal sacrifice	9	10	8	8	11	12
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Esophagus	(2)					
Muscularis, epithelium, necrosis, diffuse	1 (50%)					
Muscularis, periesophageal tissue, inflammation, chronic active, focal	1 (50%)					
Periesophageal tissue, foreign body, focal	1 (50%)					
Intestine small, duodenum	(13)	(14)	(11)	(12)	(13)	(15)
Serosa, inflammation, acute, focal					1 (8%)	
Liver	(15)	(15)	(15)	(15)	(14)	(15)
Hematopoietic cell proliferation	1 (7%)		1 (7%)	1 (7%)		
Infiltration cellular, diffuse, polymorphonuclear					1 (7%)	1 (7%)
Inflammation, acute, focal			1 (7%)		1 (7%)	
Inflammation, chronic active, diffuse			1 (7%)			
Necrosis, chronic active, focal					1 (7%)	
Necrosis, focal	2 (13%)	2 (13%)	3 (20%)	2 (13%)	1 (7%)	2 (13%)
Centrilobular, vacuolization cytoplasmic						1 (7%)
Hepatocyte, degeneration, focal			1 (7%)			
Hepatocyte, necrosis, focal	1 (7%)	1 (7%)	3 (20%)	5 (33%)	3 (21%)	4 (27%)
Hepatocyte, vacuolization cytoplasmic, diffuse	4 (27%)	5 (33%)	3 (20%)		4 (29%)	3 (20%)
Hepatocyte, vacuolization cytoplasmic, focal	2 (13%)	2 (13%)	1 (7%)	4 (27%)	2 (14%)	3 (20%)
Hepatocyte, periportal, vacuolization cytoplasmic			1 (7%)			2 (13%)
Hepatocyte, centrilobular, vacuolization cytoplasmic						1 (7%)
Serosa, inflammation, acute, focal					1 (7%)	
Pancreas	(1)	(1)	(1)		(3)	
Inflammation, acute, diffuse					1 (33%)	
Necrosis, focal		1 (100%)				
Acinus, atrophy, diffuse			1 (100%)			
Stomach, forestomach	(14)	(15)	(12)	(13)	(14)	(15)
Muscularis, serosa, inflammation, acute, focal					1 (7%)	
Stomach, glandular	(13)	(14)	(11)	(13)	(13)	(15)
Serosa, inflammation, acute, focal					1 (8%)	

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Cardiovascular System						
Blood vessel	(1)					
Aorta, inflammation, chronic active, focal	1 (100%)					
Heart		(1)		(1)		
Atrium, thrombosis, focal				1 (100%)		
Endocrine System						
Adrenal cortex	(13)	(14)	(12)	(13)	(12)	(15)
Atrophy	4 (31%)	6 (43%)	9 (75%)	3 (23%)	11 (92%)	4 (27%)
Mineralization, focal						1 (7%)
Subcapsular, hyperplasia, focal	2 (15%)	4 (29%)	3 (25%)	9 (69%)	5 (42%)	6 (40%)
Zona reticularis, hyperplasia, diffuse					1 (8%)	
Zona reticularis, infiltration cellular, focal, lymphocyte					1 (8%)	
Zona reticularis, diffuse	6 (46%)	8 (57%)	6 (50%)	10 (77%)	9 (75%)	12 (80%)
Zona reticularis, vacuolization cytoplasmic, focal	6 (46%)	4 (29%)	5 (42%)	2 (15%)	1 (8%)	3 (20%)
Thyroid gland	(13)	(13)	(12)	(14)	(12)	(14)
Ectopic thymus			1 (8%)		1 (8%)	
Follicle, cyst, focal	1 (8%)				1 (8%)	
General Body System						
None						
Genital System						
Ovary	(13)	(15)	(14)	(14)	(13)	(15)
Angiectasis, focal		1 (7%)				
Atrophy		2 (13%)	2 (14%)		1 (8%)	
Cyst			1 (7%)		1 (8%)	
Cyst, focal						1 (7%)
Degeneration	1 (8%)		2 (14%)	1 (7%)	1 (8%)	1 (7%)
Inflammation, acute, diffuse					1 (8%)	
Inflammation, chronic active, diffuse					1 (8%)	
Inflammation, diffuse						1 (7%)
Bilateral, inflammation, chronic active, diffuse	1 (8%)					
Bilateral, periovarian tissue, cyst					1 (8%)	
Corpus luteum, inflammation, chronic active, focal			1 (7%)			
Periovarian tissue, inflammation, acute, focal						1 (7%)
Periovarian tissue, inflammation, chronic active, diffuse	1 (8%)					
Periovarian tissue, inflammation, chronic active, focal		1 (7%)		1 (7%)		
Periovarian tissue, rete ovarii, inflammation, acute, focal			1 (7%)		1 (8%)	

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Genital System (continued)						
Uterus	(13)	(15)	(13)	(14)	(12)	(15)
Atrophy					1 (8%)	
Hydrometra		1 (7%)		2 (14%)		
Inflammation, acute, diffuse		1 (7%)				
Inflammation, acute, focal			1 (8%)			
Inflammation, chronic active, diffuse			1 (8%)			
Endometrium, hyperplasia, cystic	11 (85%)	10 (67%)	8 (62%)	4 (29%)	10 (83%)	13 (87%)
Endometrium, inflammation, acute, focal	1 (8%)					
Serosa, inflammation, acute, focal					1 (8%)	
Vagina			(1)			
Inflammation, chronic active, diffuse			1 (100%)			
Hematopoietic System						
Lymph node					(1)	
Iliac, hyperplasia					1 (100%)	
Renal, hyperplasia					1 (100%)	
Lymph node, mandibular	(14)	(15)	(12)	(14)	(12)	(15)
Hyperplasia	2 (14%)	1 (7%)			2 (17%)	2 (13%)
Inflammation, acute, focal			1 (8%)			
Necrosis, focal						1 (7%)
Lymph node, mesenteric	(13)	(14)	(12)	(15)	(12)	(15)
Hyperplasia	1 (8%)					
Lymph node, mediastinal	(14)	(15)	(14)	(14)	(12)	(14)
Atrophy		1 (7%)				
Hyperplasia			1 (7%)		1 (8%)	
Inflammation, diffuse						1 (7%)
Spleen	(15)	(14)	(15)	(15)	(13)	(15)
Hematopoietic cell proliferation	13 (87%)	11 (79%)	15 (100%)	13 (87%)	12 (92%)	14 (93%)
Pigmentation	10 (67%)	11 (79%)	10 (67%)	14 (93%)	11 (85%)	14 (93%)
Capsule, inflammation, acute, focal					2 (15%)	
Lymphoid follicle, depletion cellular	1 (7%)	1 (7%)		1 (7%)		
Thymus	(13)	(13)	(12)	(13)	(11)	(15)
Atrophy, diffuse	5 (38%)	3 (23%)		2 (15%)		1 (7%)
Atrophy, focal		2 (15%)	1 (8%)	3 (23%)	2 (18%)	6 (40%)
Integumentary System						
Mammary gland	(1)					
Dilatation, focal	1 (100%)					
Skin	(15)	(15)	(15)	(15)	(14)	(15)
Dermis, fibrosis, focal			1 (7%)			
Dermis, subcutaneous tissue, inflammation, chronic active, diffuse			1 (7%)			
Epidermis, hyperplasia, focal			1 (7%)			
Musculoskeletal System						
None						
Nervous System						
None						

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Respiratory System						
Lung	(14)	(15)	(15)	(15)	(14)	(15)
Alveolus, hemorrhage, focal				1 (7%)		1 (7%)
Alveolus, hyperplasia, focal, histiocytic			1 (7%)			
Alveolus, inflammation, chronic, focal					1 (7%)	
Arteriole, necrosis, focal			1 (7%)			
Mediastinum, inflammation, acute, focal	1 (7%)				1 (7%)	
Perivascular, infiltration cellular, lymphocyte			1 (7%)		1 (7%)	1 (7%)
Serosa, mediastinum, inflammation, chronic active, focal		1 (7%)				
Special Senses System						
None						
Urinary System						
Kidney	(15)	(14)	(14)	(15)	(13)	(15)
Infiltration cellular, focal, lymphocyte						1 (7%)
Inflammation, chronic active, focal			1 (7%)		1 (8%)	
Necrosis, focal			1 (7%)			
Bilateral, inflammation, acute, focal	1 (7%)					1 (7%)
Bilateral, nephropathy, diffuse	1 (7%)					
Capsule, inflammation, acute, focal			1 (7%)			
Cortex, inflammation, acute, focal		1 (7%)				
Cortex, pelvis, inflammation, chronic active, focal	1 (7%)					
Papilla, inflammation, acute, focal				1 (7%)		
Renal tubule, degeneration, focal		1 (7%)				
Renal tubule, dilatation, diffuse	1 (7%)	1 (7%)	1 (7%)			
Renal tubule, dilatation, focal				2 (13%)	1 (8%)	

APPENDIX C
SUMMARY OF LESIONS IN C57BL/6 MICE IN THE 40-WEEK GAVAGE STUDY OF ALLYL BROMIDE

TABLE C1
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Male C57BL/6 Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	8 mg/kg
Disposition Summary		
Animals initially in study	15	15
Early death		
Moribund	1	
Survivors		
Terminal sacrifice	14	15
Animals examined microscopically	15	15
Alimentary System		
Liver	(15)	(15)
Infiltration cellular, focal, lymphocyte	1 (7%)	
Hepatocyte, vacuolization cytoplasmic, diffuse	4 (27%)	9 (60%)
Hepatocyte, periportal, vacuolization cytoplasmic	2 (13%)	1 (7%)
Hepatocyte, centrilobular, vacuolization cytoplasmic	2 (13%)	
Stomach, forestomach	(15)	(15)
Serosa, necrosis, focal		1 (7%)
Cardiovascular System		
None		
Endocrine System		
Adrenal cortex	(15)	(15)
Atrophy	4 (27%)	3 (20%)
Hypertrophy, focal	1 (7%)	
Subcapsular, hyperplasia, focal		3 (20%)
Thyroid gland	(14)	(15)
Follicle, inflammation, acute, focal	1 (7%)	
General Body System		
None		
Genital System		
Testes	(15)	(15)
Germinal epithelium, degeneration, diffuse		2 (13%)
Hematopoietic System		
Spleen	(15)	(15)
Hematopoietic cell proliferation	4 (27%)	1 (7%)
Thymus	(15)	(15)
Atrophy, diffuse	1 (7%)	

TABLE C1
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Male C57BL/6 Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	8 mg/kg
Integumentary System		
None		
Musculoskeletal System		
None		
Nervous System		
None		
Respiratory System		
Lung	(15)	(15)
Perivascular, infiltration cellular, lymphocyte	1 (7%)	
Special Senses System		
None		
Urinary System		
Kidney	(15)	(15)
Pelvis, infiltration cellular, focal, lymphocyte	3 (20%)	2 (13%)
Renal tubule, dilatation, diffuse	1 (7%)	

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

TABLE C2
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female C57BL/6 Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	8 mg/kg
Disposition Summary		
Animals initially in study	15	15
Early deaths		
Moribund		2
Natural death		1
Survivors		
Terminal sacrifice	15	12
Animals examined microscopically	15	15
Alimentary System		
Intestine small, jejunum	(15)	(15)
Degeneration, focal		1 (7%)
Sarcoma		1 (7%)
Liver	(15)	(15)
Infiltration cellular, focal, lymphocyte	2 (13%)	6 (40%)
Hepatocyte, periportal, vacuolization, cytoplasmic	9 (60%)	6 (40%)
Cardiovascular System		
None		
Endocrine System		
Adrenal cortex	(15)	(14)
Subcapsular, hyperplasia, focal	14 (93%)	10 (71%)
Thyroid gland	(15)	(15)
Ectopic thymus		1 (7%)
General Body System		
Tissue NOS		(1)
Sarcoma		1 (100%)
Genital System		
Uterus	(15)	(15)
Endometrium, hyperplasia, cystic	13 (87%)	12 (80%)
Hematopoietic System		
Lymph node		(1)
Hyperplasia		1 (100%)
Inguinal, hyperplasia		1 (100%)
Lymph node, mandibular	(15)	(15)
Hyperplasia	1 (7%)	3 (20%)
Lymph node, mediastinal	(15)	(11)
Hyperplasia		1 (9%)
Spleen	(15)	(14)
Hematopoietic cell proliferation	9 (60%)	12 (86%)
Pigmentation	7 (47%)	4 (29%)
Thymus	(15)	(14)
Atrophy, diffuse		2 (14%)
Atrophy, focal		1 (7%)

TABLE C2
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female C57BL/6 Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	8 mg/kg
Integumentary System		
Skin	(15)	(15)
Epidermis, ulcer, chronic active, diffuse		1 (7%)
Epidermis, control, inflammation, chronic active, diffuse		1 (7%)
Epidermis, control, ulcer, chronic active, diffuse		1 (7%)
Musculoskeletal System		
None		
Nervous System		
None		
Respiratory System		
Lung	(15)	(15)
Alveolus, inflammation, chronic active, focal		1 (7%)
Perivascular, infiltration cellular, lymphocyte		2 (13%)
Special Senses System		
None		
Urinary System		
Kidney	(15)	(15)
Mineralization, focal	1 (7%)	
Pelvis, infiltration cellular, focal, lymphocyte		2 (13%)
Renal tubule, dilatation, focal	2 (13%)	4 (27%)
Neoplasm Summary		
Total animals with primary neoplasms[b]		1
Total primary neoplasms		2
Total animals with malignant neoplasms		1
Total malignant neoplasms		2

[a] Number of animals examined microscopically at site and number of animals with lesion
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

APPENDIX D
SUMMARY OF LESIONS IN P53 HAPLOINSUFFICIENT MICE IN THE 40-WEEK GAVAGE STUDY OF ALLYL BROMIDE

TABLE D1
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund					1	
Natural deaths		1			1	
Survivors						
Terminal sacrifice	15	14	15	15	13	15
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Stomach, forestomach	(15)	(15)	(15)	(15)	(14)	(15)
Squamous cell papilloma				1 (7%)		
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
Skeletal muscle			(1)			
Rhabdomyosarcoma			1 (100%)			
Nervous System						
None						

TABLE D1
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(15)
Alveolar/bronchiolar adenoma				1 (7%)	1 (7%)	
Special Senses System						
None						
Urinary System						
None						
Neoplasm Summary						
Total animals with primary neoplasms[b]			1	2	1	
Total primary neoplasms			1	2	1	
Total animals with benign neoplasms				2	1	
Total benign neoplasms				2	1	
Total animals with malignant neoplasms			1			
Total malignant neoplasms			1			

[a] Number of animals examined microscopically at site and number of animals with neoplasm
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund					1	
Natural deaths		1			1	
Survivors						
Terminal sacrifice	15	14	15	15	13	15
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(15)	(14)	(15)
Basophilic focus					1 (7%)	
Infiltration cellular, focal, lymphocyte			1 (7%)			2 (13%)
Tension lipidosis						1 (7%)
Hepatocyte, necrosis, focal	1 (7%)					
Hepatocyte, vacuolization cytoplasmic, diffuse	5 (33%)	7 (47%)	11 (73%)	7 (47%)	8 (57%)	6 (40%)
Hepatocyte, vacuolization cytoplasmic, focal				1 (7%)		1 (7%)
Hepatocyte, periportal, vacuolization cytoplasmic	4 (27%)	2 (13%)	2 (13%)	1 (7%)	2 (14%)	2 (13%)
Hepatocyte, centrilobular, vacuolization cytoplasmic	3 (20%)	1 (7%)		2 (13%)	2 (14%)	3 (20%)
Salivary glands					(1)	
Infiltration cellular, focal, lymphocyte					1 (100%)	
Stomach, forestomach	(15)	(15)	(15)	(15)	(14)	(15)
Epithelium, ulcer, focal				1 (7%)		
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(15)	(15)	(15)	(15)	(14)	(15)
Atrophy		1 (7%)	1 (7%)			
Degeneration, diffuse				1 (7%)		1 (7%)
Hypertrophy, focal	1 (7%)					
Subcapsular, hyperplasia, focal	1 (7%)	1 (7%)	1 (7%)	1 (7%)		1 (7%)
Adrenal medulla	(15)	(15)	(15)	(15)	(14)	(15)
Degeneration, diffuse				1 (7%)		
Thyroid gland	(15)	(15)	(14)	(15)	(14)	(15)
Follicular cell, hyperplasia, focal			1 (7%)			

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

TABLE D2
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
General System						
Tissue NOS				(1)		
Abdominal, fat, necrosis, focal				1 (100%)		
Genital System						
Epididymis	(15)	(15)	(15)	(15)	(14)	(15)
Granuloma sperm, focal	1 (7%)					
Necrosis, focal		1 (7%)				
Testes	(15)	(15)	(15)	(15)	(14)	(15)
Degeneration, diffuse						1 (7%)
Inflammation, focal, granulomatous	1 (7%)					
Mineralization, focal		1 (7%)				
Germinal epithelium, degeneration, diffuse					1 (7%)	
Germinal epithelium, degeneration, focal		1 (7%)			2 (14%)	
Hematopoietic System						
Lymph node, mesenteric	(13)	(14)	(12)	(14)	(12)	(14)
Hyperplasia					1 (8%)	
Spleen	(15)	(15)	(15)	(15)	(14)	(15)
Hematopoietic cell proliferation		1 (7%)	2 (13%)	2 (13%)	2 (14%)	3 (20%)
Pigmentation	1 (7%)	1 (7%)				
Integumentary System						
Skin	(15)	(15)	(15)	(15)	(14)	(15)
Epidermis, control, hyperplasia, focal						1 (7%)
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(15)
Alveolar epithelium, inflammation, chronic focal				1 (7%)		
Perivascular, alveolus, inflammation, chronic active, focal	1 (7%)					
Special Senses System						
None						

TABLE D2
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Urinary System						
Kidney	(15)	(15)	(15)	(15)	(14)	(15)
Cyst			1 (7%)			
Infiltration cellular, focal, lymphocyte				1 (7%)		
Pelvis, infiltration cellular, focal, lymphocyte	2 (13%)		2 (13%)		2 (14%)	1 (7%)
Renal tubule, dilatation, diffuse			1 (7%)			

TABLE D3
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund	2	1	2			1
Natural deaths				1		1
Survivors						
Terminal sacrifice	13	14	13	14	15	13
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Liver	(15)	(15)	(15)	(14)	(15)	(15)
Lymphoma malignant	1 (7%)	1 (7%)				1 (7%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(15)	(15)	(15)	(14)	(15)	(15)
Lymphoma malignant						1 (7%)
Adrenal medulla	(15)	(15)	(15)	(14)	(15)	(15)
Lymphoma malignant						1 (7%)
General Body System						
Tissue NOS	(1)			(1)		(1)
Sarcoma				1 (100%)		
Abdominal, sarcoma						1 (100%)
Pelvic, fibrosarcoma	1 (100%)					
Genital System						
None						
Hematopoietic System						
Lymph node		(1)			(1)	
Lymphoma malignant		1 (100%)				
Lymph node, mandibular	(15)	(14)	(15)	(14)	(15)	(13)
Lymphoma malignant		1 (7%)				
Lymph node, mesenteric	(15)	(14)	(15)	(12)	(15)	(15)
Lymphoma malignant		1 (7%)				
Lymph node, mediastinal	(15)	(14)	(15)	(14)	(15)	(14)
Lymphoma malignant		1 (7%)				1 (7%)
Spleen	(15)	(15)	(15)	(14)	(15)	(15)
Lymphoma malignant	1 (7%)	1 (7%)				1 (7%)
Thymus	(14)	(14)	(14)	(14)	(15)	(14)
Lymphoma malignant	1 (7%)					1 (7%)

TABLE D3
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Integumentary System						
Skin	(15)	(15)	(15)	(14)	(15)	(15)
Subcutaneous tissue, osteosarcoma	1 (7%)					
Musculoskeletal System						
Bone	(2)	(1)	(1)			
Osteosarcoma			1 (100%)			
Mandible, osteosarcoma		1 (100%)				
Vertebra, osteosarcoma	1 (50%)					
Nervous System						
None						
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(15)
Lymphoma malignant						1 (7%)
Osteosarcoma, metastatic, skin	1 (7%)					
Special Senses System						
None						
Urinary System						
None						
Systemic Lesions						
Multiple organs[b]	(15)	(15)	(15)	(15)	(15)	(15)
Lymphoma malignant	1 (7%)	1 (7%)				1 (7%)
Neoplasm Summary						
Total animals with primary neoplasms[c]	4	2	1	1		2
Total primary neoplasms	4	2	1	1		2
Total animals with malignant neoplasms	4	2	1	1		2
Total malignant neoplasms	4	2	1	1		2
Total animals with metastatic neoplasms	1					
Total metastatic neoplasms	1					

[a] Number of animals examined microscopically at site and number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Disposition Summary						
Animals initially in study	15	15	15	15	15	15
Early deaths						
Moribund	2	1	2			1
Natural deaths				1		1
Survivors						
Terminal sacrifice	13	14	13	14	15	13
Animals examined microscopically	15	15	15	15	15	15
Alimentary System						
Intestine small, duodenum	(15)	(15)	(15)	(14)	(15)	(14)
Polyp inflammatory, focal						1 (7%)
Liver	(15)	(15)	(15)	(14)	(15)	(15)
Infiltration cellular, focal, lymphocyte	3 (20%)	2 (13%)	4 (27%)	6 (43%)	7 (47%)	2 (13%)
Necrosis, focal	1 (7%)					
Tension lipidosis						1 (7%)
Hepatocyte, necrosis, focal				1 (7%)	1 (7%)	1 (7%)
Hepatocyte, vacuolization cytoplasmic, focal					1 (7%)	2 (13%)
Hepatocyte, periportal, vacuolization cytoplasmic	8 (53%)	8 (53%)	8 (53%)	6 (43%)	3 (20%)	5 (33%)
Salivary glands						(1)
Infiltration cellular, focal, lymphocyte						1 (100%)
Stomach, forestomach	(15)	(15)	(15)	(14)	(15)	(14)
Hyperkeratosis, focal					1 (7%)	
Epithelium, hyperplasia, focal					1 (7%)	
Muscularis, inflammation, acute, focal					1 (7%)	
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(15)	(15)	(15)	(14)	(15)	(15)
Subcapsular, hyperplasia, focal	13 (87%)	14 (93%)	15 (100%)	12 (86%)	12 (80%)	13 (87%)
Parathyroid gland			(1)			
Cyst			1 (100%)			
Pituitary gland	(14)	(14)	(15)	(13)	(15)	(13)
Cyst, focal			1 (7%)			
Thyroid gland	(14)	(15)	(15)	(14)	(14)	(15)
Ectopic thymus	1 (7%)				1 (7%)	1 (7%)
General Body System						
None						

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

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Genital System						
Ovary	(15)	(15)	(15)	(14)	(15)	(14)
Cyst	1 (7%)					
Degeneration	1 (7%)					
Uterus	(15)	(15)	(15)	(14)	(15)	(14)
Inflammation, acute					1 (7%)	
Thrombosis, focal						1 (7%)
Endometrium, hyperplasia, cystic	13 (87%)	13 (87%)	14 (93%)	13 (93%)	14 (93%)	14 (100%)
Hematopoietic System						
Lymph node		(1)			(1)	
Hyperplasia					1 (100%)	
Lymph node, mandibular	(15)	(14)	(15)	(14)	(15)	(13)
Hyperplasia			1 (7%)	1 (7%)	1 (7%)	
Lymph node, mesenteric	(15)	(14)	(15)	(12)	(15)	(15)
Hyperplasia					1 (7%)	
Lymph node, mediastinal	(15)	(14)	(15)	(14)	(15)	(14)
Hyperplasia			1 (7%)			
Spleen	(15)	(15)	(15)	(14)	(15)	(15)
Hematopoietic cell proliferation	8 (53%)	6 (40%)	7 (47%)	10 (71%)	9 (60%)	7 (47%)
Hyperplasia, lymphoid	1 (7%)	1 (7%)				
Pigmentation	4 (27%)	8 (53%)	4 (27%)	2 (14%)	2 (13%)	2 (13%)
Lymphoid follicle, depletion cellular, diffuse		1 (7%)				1 (7%)
Lymphoid follicle, hyperplasia		1 (7%)				
Thymus	(14)	(14)	(14)	(14)	(15)	(14)
Atrophy, diffuse	1 (7%)				2 (13%)	1 (7%)
Atrophy, focal		1 (7%)				2 (14%)
Hyperplasia, focal			1 (7%)			
Integumentary System						
Skin	(15)	(15)	(15)	(14)	(15)	(15)
Epidermis, hyperplasia, diffuse	1 (7%)					
Epidermis, hyperplasia, focal			2 (13%)		1 (7%)	
Subcutaneous tissue, edema, diffuse	1 (7%)					
Musculoskeletal System						
Bone	(2)	(1)	(1)			
Metatarsal, fracture	1 (50%)					
Nervous System						
None						

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Respiratory System						
Lung	(15)	(15)	(15)	(15)	(15)	(15)
Alveolar epithelium, hyperplasia, focal				1 (7%)		
Alveolus, inflammation, chronic active, focal					1 (7%)	
Perivascular, infiltration cellular, lymphocyte					1 (7%)	2 (13%)
Special Senses System						
None						
Urinary System						
Kidney	(15)	(15)	(15)	(14)	(15)	(15)
Pelvis, infiltration cellular, focal, lymphocyte		1 (7%)				2 (13%)
Renal tubule, dilatation, focal	4 (27%)		3 (20%)	1 (7%)	2 (13%)	2 (13%)

APPENDIX E
GENETIC TOXICOLOGY

TABLE E1
Mutagenicity of Allyl Bromide in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+30% rat S9 Trial 1	+30% rat S9 Trial 2
TA100	0	117 ± 3.8	123 ± 8.1	114 ± 5.5	119 ± 3.2	111 ± 3.9	135 ± 8.5
	3			133 ± 8.4	126 ± 3.3	118 ± 0.9	139 ± 8.9
	10	115 ± 0.9	126 ± 5.6	162 ± 3.9	143 ± 7.1	122 ± 1.8	150 ± 5.9
	33	138 ± 4.1	136 ± 3.6	182 ± 11.3	165 ± 4.0	150 ± 7.6	166 ± 5.5
	100	183 ± 3.1	171 ± 9.4	200 ± 10.0	186 ± 6.1	169 ± 3.8	187 ± 4.7
	333	411 ± 23.4	388 ± 7.3	192 ± 3.4	214 ± 7.5	228 ± 15.0	227 ± 8.6
	666		352 ± 13.8				
	1,000	109 ± 5.2[c]					
Trial summary		Positive	Positive	Weakly Positive	Weakly Positive	Weakly Positive	Weakly Positive
Positive control[d]		888 ± 23.3	872 ± 16.0	543 ± 11.6	508 ± 7.8	492 ± 8.0	470 ± 17.9
TA98	0	13 ± 2.3		17 ± 1.0		22 ± 1.7	
	3			19 ± 2.9		15 ± 1.9	
	10	11 ± 0.6		16 ± 1.3		18 ± 1.8	
	33	12 ± 2.0		18 ± 4.2		21 ± 3.7	
	100	9 ± 0.9		21 ± 2.6		13 ± 1.9	
	333	10 ± 0.3		18 ± 0.7		19 ± 0.9	
	1,000	5 ± 0.3[c]					
Trial summary		Negative		Negative		Negative	
Positive control		334 ± 22.1		440 ± 15.4		329 ± 14.0	

[a] The study was performed at SRI International. The detailed protocol is presented by Zeiger *et al*. (1992). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100) and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of FVB/N Mice Following Administration of Allyl Bromide by Gavage for 40 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P-Value[c]	PCEs[b] (%)
Male					
Corn Oil[d]	0	15	0.83 ± 0.20		2.62 ± 0.20
Allyl Bromide	8	14	1.25 ± 0.16	0.0594	2.86 ± 0.17
Female					
Corn Oil[d]	0	15	0.90 ± 0.19		2.95 ± 0.18
Allyl Bromide	8	14	1.14 ± 0.19	0.1796	2.82 ± 0.12

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990) and Witt *et al*. (2000).
[b] PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[c] Mean ± standard error
[d] Pairwise comparison with the vehicle control group; significant at $P \leq 0.05$ (ILS, 1990)
Vehicle control

TABLE E3
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of Tg.AC Hemizygous Mice Following Administration of Allyl Bromide by Gavage for 40 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P-Value[c]	PCEs[b] (%)
Male					
Corn Oil[d]	0	12	1.25 ± 0.32		4.40 ± 0.34
Allyl Bromide	0.5	9	0.89 ± 0.25	0.8659	3.92 ± 0.34
	1	9	1.17 ± 0.25	0.5959	3.71 ± 0.19
	2	12	0.92 ± 0.17	0.8665	3.94 ± 0.32
	4	6	0.92 ± 0.27	0.8116	3.82 ± 0.29
	8	11	1.27 ± 0.21	0.4726	3.41 ± 0.33
			P=0.303[e]		
Female					
Corn Oil[d]	0	9	0.44 ± 0.15		4.32 ± 0.40
Allyl Bromide	0.5	10	1.10 ± 0.18	0.0115	3.65 ± 0.17
	1	8	0.69 ± 0.19	0.1719	4.29 ± 0.34
	2	8	0.75 ± 0.30	0.1231	4.59 ± 0.67
	4	11	0.91 ± 0.21	0.0402	4.19 ± 0.52
	8	12	0.83 ± 0.17	0.0633	4.25 ± 0.25
			P=0.297[e]		

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990) and Witt *et al*. (2000).
PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; 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 E4
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of C57BL/6 Mice Following Administration of Allyl Bromide by Gavage for 40 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P-Value[c]	PCEs[b] (%)
Male					
Corn Oil[d]	0	14	1.61 ± 0.30		3.56 ± 0.21
Allyl Bromide	8	15	0.97 ± 0.18	0.9846	4.03 ± 0.26
Female					
Corn Oil[d]	0	15	0.53 ± 0.18		4.31 ± 0.29
Allyl Bromide	8	12	0.96 ± 0.20	0.0339	3.86 ± 0.37

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990) and Witt *et al*. (2000).
PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; significant at $P \leq 0.05$ (ILS, 1990)
[d] Vehicle control

TABLE E5
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of p53 Haploinsufficient Mice Following Administration of Allyl Bromide by Gavage for 40 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P-Value[c]	PCEs[b] (%)
Male					
Corn Oil[d]	0	15	1.37 ± 0.24		3.57 ± 0.18
Allyl Bromide	0.5	14	1.64 ± 0.22	0.1952	4.61 ± 1.43
	1	15	2.53 ± 0.22	0.0006	4.13 ± 0.74
	2	15	1.50 ± 0.22	0.3330	3.79 ± 0.19
	4	13	1.92 ± 0.31	0.0515	3.95 ± 0.18
	8	15	1.63 ± 0.19	0.1994	3.49 ± 0.27
			P=0.577[e]		
Female					
Corn Oil[d]	0	13	0.58 ± 0.19		3.99 ± 0.42
Allyl Bromide	0.5	14	0.57 ± 0.12	0.5093	3.90 ± 0.46
	1	13	0.81 ± 0.22	0.1904	3.15 ± 0.21
	2	14	0.89 ± 0.23	0.1188	3.72 ± 0.27
	4	15	1.00 ± 0.21	0.0613	4.15 ± 0.35
	8	13	0.77 ± 0.17	0.2295	4.48 ± 0.69
			P=0.188[e]		

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990) and Witt *et al*. (2000). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; significant at P≤0.005 (ILS, 1990)
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

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

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for FVB/N Mice in the 2-Week Dermal Study of Allyl Bromide[a]

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt.	27.1 ± 0.9	27.2 ± 0.9	27.0 ± 0.2	27.2 ± 0.4	27.6 ± 0.5	27.5 ± 0.9
Heart						
Absolute	0.133 ± 0.005	0.131 ± 0.005	0.137 ± 0.003	0.136 ± 0.004	0.137 ± 0.005	0.140 ± 0.005
Relative	4.918 ± 0.097	4.808 ± 0.115	5.059 ± 0.101	4.989 ± 0.094	4.982 ± 0.203	5.099 ± 0.146
R. Kidney						
Absolute	0.263 ± 0.011	0.271 ± 0.019	0.271 ± 0.009	0.280 ± 0.011	0.277 ± 0.006	0.277 ± 0.010
Relative	9.695 ± 0.240	9.924 ± 0.408	10.049 ± 0.287	10.283 ± 0.289	10.041 ± 0.181	10.071 ± 0.301
Liver						
Absolute	1.620 ± 0.058	1.568 ± 0.089	1.545 ± 0.040	1.623 ± 0.064	1.660 ± 0.018	1.724 ± 0.054
Relative	59.711 ± 0.648	57.619 ± 2.141	57.237 ± 1.639	59.614 ± 1.669	60.150 ± 0.771	62.678 ± 1.194
Lung						
Absolute	0.159 ± 0.008	0.162 ± 0.009	0.163 ± 0.001	0.169 ± 0.004	0.185 ± 0.010	0.175 ± 0.012
Relative	5.862 ± 0.137	5.962 ± 0.177	6.040 ± 0.092	6.220 ± 0.130	6.714 ± 0.418	6.324 ± 0.239
R. Testis						
Absolute	0.087 ± 0.002	0.089 ± 0.003	0.089 ± 0.003	0.084 ± 0.002	0.089 ± 0.001	0.086 ± 0.002
Relative	3.223 ± 0.138	3.300 ± 0.121	3.302 ± 0.097	3.092 ± 0.103	3.237 ± 0.102	3.140 ± 0.100
Thymus						
Absolute	0.042 ± 0.002	0.041 ± 0.003	0.038 ± 0.002	0.043 ± 0.005	0.047 ± 0.003	0.046 ± 0.002
Relative	1.561 ± 0.121	1.524 ± 0.088	1.409 ± 0.084	1.590 ± 0.182	1.700 ± 0.077	1.690 ± 0.116
Female						
Necropsy body wt.	21.5 ± 0.5	21.9 ± 0.5	20.9 ± 0.4	21.3 ± 0.4	21.8 ± 0.3	21.8 ± 0.5
Heart						
Absolute	0.121 ± 0.003	0.121 ± 0.002	0.116 ± 0.002	0.121 ± 0.004	0.118 ± 0.003	0.122 ± 0.004
Relative	5.620 ± 0.088	5.557 ± 0.138	5.577 ± 0.137	5.691 ± 0.152	5.419 ± 0.152	5.608 ± 0.114
R. Kidney						
Absolute	0.177 ± 0.005	0.186 ± 0.006	0.170 ± 0.005	0.170 ± 0.004	0.174 ± 0.003	0.177 ± 0.008
Relative	8.225 ± 0.142	8.522 ± 0.219	8.139 ± 0.323	7.971 ± 0.266	8.001 ± 0.139	8.120 ± 0.337
Liver						
Absolute	1.269 ± 0.033	1.377 ± 0.050	1.251 ± 0.028	1.253 ± 0.030	1.259 ± 0.038	1.313 ± 0.063
Relative	58.888 ± 0.360	62.903 ± 1.298	59.923 ± 1.761	58.710 ± 1.033	57.789 ± 1.658	60.227 ± 2.085
Lung						
Absolute	0.156 ± 0.003	0.162 ± 0.008	0.155 ± 0.005	0.154 ± 0.006	0.151 ± 0.008	0.163 ± 0.004
Relative	7.230 ± 0.132	7.405 ± 0.360	7.404 ± 0.274	7.218 ± 0.276	6.948 ± 0.335	7.486 ± 0.185
Thymus						
Absolute	0.049 ± 0.005	0.053 ± 0.005	0.052 ± 0.002	0.051 ± 0.006	0.044 ± 0.006	0.058 ± 0.003
Relative	2.268 ± 0.223	2.427 ± 0.172	2.475 ± 0.101	2.421 ± 0.302	2.035 ± 0.292	2.691 ± 0.182

[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). Differences from the vehicle control group are not significant by Dunnett's test.

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for FVB/N Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	8 mg/kg
n	15	14
Male		
Necropsy body wt.	42.2 ± 1.0	41.8 ± 0.9
Heart		
Absolute	0.189 ± 0.003	0.185 ± 0.002
Relative	4.500 ± 0.113	4.449 ± 0.086
R. Kidney		
Absolute	0.353 ± 0.006	0.340 ± 0.007
Relative	8.421 ± 0.216	8.164 ± 0.206
Liver		
Absolute	1.914 ± 0.041	1.922 ± 0.059
Relative	45.503 ± 0.913	45.896 ± 0.706
Lung		
Absolute	0.219 ± 0.004[b]	0.239 ± 0.009
Relative	5.188 ± 0.173[b]	5.777 ± 0.297
R. Testis		
Absolute	0.090 ± 0.003	0.093 ± 0.001[b]
Relative	2.158 ± 0.098	2.255 ± 0.062[b]
Thymus		
Absolute	0.034 ± 0.004	0.030 ± 0.003
Relative	0.798 ± 0.077	0.700 ± 0.062
Female		
Necropsy body wt.	32.3 ± 1.4	32.6 ± 0.9
Heart		
Absolute	0.138 ± 0.003	0.142 ± 0.004
Relative	4.355 ± 0.133	4.353 ± 0.095
R. Kidney		
Absolute	0.202 ± 0.006	0.215 ± 0.004
Relative	6.334 ± 0.187	6.633 ± 0.194
Liver		
Absolute	1.547 ± 0.046	1.550 ± 0.033
Relative	48.439 ± 1.105	47.693 ± 0.749
Lung		
Absolute	0.213 ± 0.006	0.210 ± 0.008
Relative	6.779 ± 0.353	6.441 ± 0.176
Thymus		
Absolute	0.029 ± 0.002	0.027 ± 0.002[b]
Relative	0.910 ± 0.063	0.823 ± 0.060[b]

[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). Differences from the vehicle control group are not significant by Dunnett's test.
[b] n=13

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Male						
n	12	9	9	12	6	11
Necropsy body wt.	39.2 ± 1.5	36.5 ± 1.1	39.7 ± 1.4	36.9 ± 1.5	37.6 ± 1.5	38.3 ± 1.2
Heart						
Absolute	0.185 ± 0.005	0.175 ± 0.003	0.185 ± 0.007	0.182 ± 0.006	0.178 ± 0.006	0.184 ± 0.006
Relative	4.761 ± 0.163	4.801 ± 0.112	4.698 ± 0.198	4.976 ± 0.139	4.748 ± 0.092	4.806 ± 0.088
R. Kidney						
Absolute	0.337 ± 0.010	0.320 ± 0.014	0.324 ± 0.009	0.321 ± 0.010	0.342 ± 0.015	0.335 ± 0.010
Relative	8.660 ± 0.269	8.760 ± 0.275	8.174 ± 0.178	8.753 ± 0.171	9.158 ± 0.440	8.775 ± 0.167
Liver						
Absolute	1.946 ± 0.080	1.693 ± 0.056	1.894 ± 0.072	1.726 ± 0.069	1.855 ± 0.066	1.819 ± 0.065
Relative	49.659 ± 0.632	46.486 ± 1.390	47.786 ± 1.440	46.858 ± 0.673	49.542 ± 1.674	47.510 ± 0.702
Lung						
Absolute	0.251 ± 0.009	0.265 ± 0.026	0.324 ± 0.025*	0.247 ± 0.014	0.248 ± 0.011[b]	0.243 ± 0.012
Relative	6.462 ± 0.260	7.350 ± 0.810	8.163 ± 0.591*	6.737 ± 0.361	6.806 ± 0.526[b]	6.382 ± 0.344
R. Testis						
Absolute	0.086 ± 0.003	0.087 ± 0.003	0.075 ± 0.004*	0.083 ± 0.004	0.094 ± 0.002	0.088 ± 0.001
Relative	2.218 ± 0.084	2.374 ± 0.048	1.911 ± 0.122	2.256 ± 0.115	2.518 ± 0.124	2.315 ± 0.075
Thymus						
Absolute	0.023 ± 0.003	0.016 ± 0.002	0.024 ± 0.002	0.021 ± 0.003	0.020 ± 0.003	0.023 ± 0.002
Relative	0.562 ± 0.057	0.443 ± 0.052	0.598 ± 0.059	0.556 ± 0.068	0.533 ± 0.083	0.600 ± 0.043
Female						
n	9	10	8	8	11	12
Necropsy body wt.	30.2 ± 0.6	31.5 ± 2.5	29.7 ± 1.9	31.5 ± 1.6	30.1 ± 0.8	31.7 ± 1.3
Heart						
Absolute	0.150 ± 0.009	0.130 ± 0.006	0.130 ± 0.004	0.142 ± 0.006	0.146 ± 0.005	0.139 ± 0.004
Relative	4.947 ± 0.236	4.260 ± 0.185	4.446 ± 0.186	4.553 ± 0.236	4.863 ± 0.180	4.439 ± 0.136
R. Kidney						
Absolute	0.209 ± 0.004[c]	0.209 ± 0.012	0.207 ± 0.008	0.206 ± 0.006	0.216 ± 0.008	0.207 ± 0.005
Relative	7.023 ± 0.171[c]	6.808 ± 0.360	7.137 ± 0.441	6.645 ± 0.329	7.242 ± 0.322	6.597 ± 0.184
Liver						
Absolute	1.735 ± 0.126	1.475 ± 0.111	1.501 ± 0.032	1.604 ± 0.077	1.530 ± 0.045[d]	1.556 ± 0.059
Relative	57.291 ± 3.517	47.039 ± 0.806**	51.634 ± 2.405	51.127 ± 1.621	50.741 ± 1.170[d]	49.146 ± 0.627**
Lung						
Absolute	0.244 ± 0.014	0.196 ± 0.007*	0.226 ± 0.011	0.295 ± 0.022*	0.212 ± 0.008[d]	0.251 ± 0.011
Relative	8.097 ± 0.493	6.517 ± 0.429	7.817 ± 0.627	9.596 ± 0.974	7.012 ± 0.216[d]	7.981 ± 0.374
Thymus						
Absolute	0.024 ± 0.001	0.030 ± 0.004	0.023 ± 0.003	0.025 ± 0.003	0.025 ± 0.002	0.032 ± 0.002
Relative	0.787 ± 0.054	0.920 ± 0.067	0.774 ± 0.059	0.793 ± 0.078	0.834 ± 0.062	1.004 ± 0.046

* Significantly different ($P \leq 0.05$) from the vehicle control group by 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] n=5
[c] n=8
[d] n=10

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for C57BL/6 Mice in the 2-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Male						
n	5	5	5	5	5	2
Necropsy body wt.	22.2 ± 0.9	23.1 ± 0.6	23.2 ± 1.0	23.5 ± 0.9	23.1 ± 0.8	22.2 ± 0.4
Heart						
Absolute	0.109 ± 0.003	0.114 ± 0.004	0.111 ± 0.003	0.113 ± 0.004	0.111 ± 0.003	0.108 ± 0.005
Relative	4.920 ± 0.076	4.938 ± 0.204	4.803 ± 0.087	4.843 ± 0.218	4.797 ± 0.104	4.881 ± 0.303
R. Kidney						
Absolute	0.171 ± 0.004	0.186 ± 0.002	0.185 ± 0.003	0.179 ± 0.008	0.178 ± 0.009	0.189 ± 0.011
Relative	7.723 ± 0.218	8.071 ± 0.213	8.058 ± 0.341	7.620 ± 0.193	7.711 ± 0.178	8.520 ± 0.609
Liver						
Absolute	1.177 ± 0.088	1.313 ± 0.031	1.326 ± 0.033	1.413 ± 0.063**	1.455 ± 0.036**[b]	1.313[c]
Relative	52.764 ± 2.233	56.928 ± 1.533	57.447 ± 1.219	60.144 ± 1.554**	60.405 ± 0.696**[b]	58.356[c]
Lung						
Absolute	0.151 ± 0.008	0.162 ± 0.004	0.156 ± 0.008	0.172 ± 0.013	0.154 ± 0.005	0.158 ± 0.009
Relative	6.816 ± 0.374	7.028 ± 0.317	6.749 ± 0.133	7.358 ± 0.607	6.691 ± 0.218	7.118 ± 0.496
R. Testis						
Absolute	0.087 ± 0.003	0.077 ± 0.007	0.074 ± 0.005	0.075 ± 0.005	0.067 ± 0.003*	0.081 ± 0.011
Relative	3.904 ± 0.044	3.341 ± 0.264	3.180 ± 0.133*	3.204 ± 0.128*	2.925 ± 0.125**	3.628 ± 0.417
Thymus						
Absolute	0.047 ± 0.003	0.043 ± 0.005	0.046 ± 0.004	0.049 ± 0.003	0.050 ± 0.003	0.034 ± 0.007
Relative	2.137 ± 0.143	1.876 ± 0.209	1.985 ± 0.152	2.104 ± 0.108	2.142 ± 0.056	1.530 ± 0.292
Female						
n	5	5	5	5	5	5
Necropsy body wt.	19.8 ± 0.5	19.2 ± 1.1	20.0 ± 0.5	19.7 ± 0.7	18.7 ± 0.5	18.6 ± 0.8
Heart						
Absolute	0.108 ± 0.001[d]	0.105 ± 0.008	0.104 ± 0.003	0.102 ± 0.002	0.095 ± 0.005	0.088 ± 0.004**
Relative	5.322 ± 0.072[d]	5.488 ± 0.232	5.201 ± 0.073	5.199 ± 0.135	5.060 ± 0.193	4.722 ± 0.077*
R. Kidney						
Absolute	0.139 ± 0.006	0.137 ± 0.003[d]	0.144 ± 0.005	0.140 ± 0.006	0.136 ± 0.002	0.142 ± 0.006
Relative	6.992 ± 0.169	7.525 ± 0.507[d]	7.202 ± 0.082	7.122 ± 0.250	7.281 ± 0.175	7.649 ± 0.169
Liver						
Absolute	1.042 ± 0.061	0.992 ± 0.065	1.001 ± 0.040	1.065 ± 0.046	1.013 ± 0.067[b]	—[e]
Relative	52.400 ± 1.952	51.858 ± 3.063	49.963 ± 1.703	54.116 ± 2.084	54.057 ± 1.673[b]	—[e]
Lung						
Absolute	0.178 ± 0.020	0.149 ± 0.006	0.155 ± 0.006	0.147 ± 0.004	0.142 ± 0.008	0.146 ± 0.003
Relative	9.031 ± 1.096	7.819 ± 0.317	7.709 ± 0.143	7.502 ± 0.337	7.629 ± 0.503	7.878 ± 0.187
Thymus						
Absolute	0.061 ± 0.015	0.075 ± 0.006	0.068 ± 0.005	0.079 ± 0.005	0.065 ± 0.004	0.049 ± 0.003
Relative	3.071 ± 0.716	3.958 ± 0.389	3.392 ± 0.239	4.006 ± 0.231	3.454 ± 0.190	2.638 ± 0.236

* Significantly different (P≤0.05) from the vehicle control group by William's or Dunnett's test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=3
[c] n=1
[d] n=4
[e] n=0

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for C57BL/6 Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	8 mg/kg
Male		
n	14	15
Necropsy body wt.	46.2 ± 1.8	46.4 ± 1.4
Heart		
Absolute	0.173 ± 0.006	0.164 ± 0.004
Relative	3.765 ± 0.108	3.580 ± 0.144
R. Kidney		
Absolute	0.240 ± 0.006	0.238 ± 0.004
Relative	5.261 ± 0.137	5.204 ± 0.211
Liver		
Absolute	2.050 ± 0.141	1.956 ± 0.083
Relative	43.808 ± 1.601	42.082 ± 0.989
Lung		
Absolute	0.211 ± 0.005	0.210 ± 0.006
Relative	4.686 ± 0.259	4.605 ± 0.217
R. Testis		
Absolute	0.094 ± 0.002	0.091 ± 0.002[b]
Relative	2.068 ± 0.083	1.972 ± 0.079[b]
Thymus		
Absolute	0.054 ± 0.005	0.061 ± 0.008
Relative	1.165 ± 0.097	1.321 ± 0.150
Female		
n	15	12
Necropsy body wt.	39.4 ± 1.3	37.6 ± 1.6
Heart		
Absolute	0.148 ± 0.004[b]	0.148 ± 0.004
Relative	3.812 ± 0.145[b]	3.995 ± 0.168
R. Kidney		
Absolute	0.190 ± 0.003	0.190 ± 0.005
Relative	4.882 ± 0.172	5.112 ± 0.178
Liver		
Absolute	1.554 ± 0.042	1.489 ± 0.041
Relative	39.741 ± 1.022	39.921 ± 0.850
Lung		
Absolute	0.224 ± 0.008	0.218 ± 0.007
Relative	5.719 ± 0.190	5.937 ± 0.382
Thymus		
Absolute	0.055 ± 0.004[b]	0.053 ± 0.005
Relative	1.429 ± 0.110[b]	1.443 ± 0.156

[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). Differences from the vehicle control group are not significant by Dunnett's test.

[b] n=14

TABLE F6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide[a]

	Vehicle Control	0.5 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg
Male						
n	15	14	15	15	13	15
Necropsy body wt.	47.7 ± 1.6	49.8 ± 1.6	49.9 ± 1.0	48.4 ± 1.8	50.3 ± 2.1	50.4 ± 1.1
Heart						
Absolute	0.169 ± 0.003[b]	0.183 ± 0.007	0.173 ± 0.003	0.173 ± 0.006	0.186 ± 0.005	0.182 ± 0.005
Relative	3.589 ± 0.086[b]	3.678 ± 0.101	3.477 ± 0.082	3.600 ± 0.120	3.780 ± 0.200	3.626 ± 0.104
R. Kidney						
Absolute	0.256 ± 0.006	0.272 ± 0.010	0.269 ± 0.010	0.259 ± 0.011	0.276 ± 0.006	0.267 ± 0.005
Relative	5.419 ± 0.188	5.492 ± 0.189	5.432 ± 0.231	5.432 ± 0.279	5.613 ± 0.292	5.315 ± 0.096
Liver						
Absolute	2.017 ± 0.103	2.228 ± 0.173[c]	1.954 ± 0.094	2.103 ± 0.162	2.273 ± 0.159	2.144 ± 0.127
Relative	42.171 ± 1.424	44.148 ± 1.975[c]	39.022 ± 1.356	42.733 ± 1.909	44.562 ± 1.793	42.147 ± 1.699
Lung						
Absolute	0.223 ± 0.012	0.219 ± 0.007	0.222 ± 0.010	0.207 ± 0.007	0.234 ± 0.012	0.198 ± 0.004
Relative	4.782 ± 0.344	4.480 ± 0.245	4.475 ± 0.220	4.367 ± 0.201	4.758 ± 0.322	3.956 ± 0.100
R. Testis						
Absolute	0.115 ± 0.002	0.119 ± 0.002	0.119 ± 0.002	0.115 ± 0.004	0.117 ± 0.003	0.119 ± 0.002[b]
Relative	2.439 ± 0.070	2.413 ± 0.078	2.403 ± 0.055	2.396 ± 0.081	2.362 ± 0.093	2.385 ± 0.046[b]
Thymus						
Absolute	0.043 ± 0.003	0.052 ± 0.006	0.047 ± 0.005	0.048 ± 0.005	0.055 ± 0.007	0.054 ± 0.005
Relative	0.913 ± 0.069	1.039 ± 0.103	0.948 ± 0.085	0.992 ± 0.103	1.114 ± 0.139	1.080 ± 0.104
Female						
n	13	14	13	14	15	13
Necropsy body wt.	43.1 ± 2.2	43.5 ± 1.5	42.7 ± 1.6	40.1 ± 1.7	38.1 ± 1.7	44.8 ± 1.8
Heart						
Absolute	0.151 ± 0.004	0.151 ± 0.002	0.155 ± 0.004	0.149 ± 0.004	0.154 ± 0.004	0.155 ± 0.004
Relative	3.571 ± 0.124	3.527 ± 0.121	3.680 ± 0.155	3.789 ± 0.151	4.114 ± 0.141*	3.490 ± 0.101
R. Kidney						
Absolute	0.202 ± 0.005	0.199 ± 0.003	0.194 ± 0.005	0.191 ± 0.006	0.207 ± 0.003	0.194 ± 0.006
Relative	4.803 ± 0.194	4.627 ± 0.148	4.616 ± 0.182	4.830 ± 0.168	5.562 ± 0.235*	4.362 ± 0.119
Liver						
Absolute	1.695 ± 0.069	1.721 ± 0.074	1.554 ± 0.053	1.496 ± 0.052	1.593 ± 0.055	1.606 ± 0.043[d]
Relative	39.730 ± 1.165	40.116 ± 2.226	36.739 ± 1.269	37.540 ± 0.749	42.265 ± 1.167	37.165 ± 1.174[d]
Lung						
Absolute	0.219 ± 0.009	0.209 ± 0.003[c]	0.221 ± 0.009	0.214 ± 0.008	0.232 ± 0.007	0.205 ± 0.004[d]
Relative	5.282 ± 0.433	4.826 ± 0.150[c]	5.267 ± 0.290	5.449 ± 0.275	6.209 ± 0.273	4.609 ± 0.170[d]
Thymus						
Absolute	0.050 ± 0.004	0.047 ± 0.004	0.050 ± 0.005	0.054 ± 0.004	0.050 ± 0.004	0.051 ± 0.006
Relative	1.182 ± 0.117	1.102 ± 0.105	1.190 ± 0.122	1.374 ± 0.095	1.326 ± 0.112	1.143 ± 0.140

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

[a] Organ weights (absolute weights) and body weights are given as grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

[b] n=14

[c] n=13

[d] n=12

APPENDIX G
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Allyl Bromide

Allyl bromide was obtained from Fluka Chemical Corporation (Buchs, Switzerland) in one lot (330638) and from Aldrich Chemical Co. in one lot (03614HN). Lot 330638 was used in the 2-week studies and lot 03614HN was used in the 40-week studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) and the study laboratory, BioReliance (Rockville, MD). Reports on analyses performed in support of the allyl bromide studies are on file at the National Institute of Environmental Health Sciences.

Both lots of allyl bromide, a clear, colorless liquid, were identified by the analytical chemistry laboratory using infrared (IR) and proton nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using IR spectroscopy. All IR and NMR spectra were consistent with the literature spectra (*Aldrich*, 1985, 1993) and spectra of a reference standard of the same lot. Representative IR and NMR spectra are presented in Figures G1 and G2.

The purity of each lot was determined by the analytical chemistry laboratory using gas chromatography (GC) by system A and by the study laboratory using GC by system B (Table G1). For lot 330638, GC by system A indicated one major peak and five impurities with a combined peak area of 0.7% relative to the total peak area. GC by system B indicated one major peak and three impurities with a combined peak area of less than 0.5%. The relative purity was 102% when compared to a reference standard from the same lot. The overall purity of lot 330638 was greater than 99%. For lot 03614HN, GC by system A indicated one major peak and four impurities with a combined peak area of 0.45% relative to the total peak area. GC by system B indicated one major peak and three impurities with a total combined area less than 0.3% of the total peak area. The relative purity was 102% when compared to a reference standard from the same lot. The overall purity of lot 03614HN was greater than 99%.

During the 40-week studies, additional purity analyses were performed by the study laboratory at 26 weeks and at the end of the study using GC by system B. To ensure stability, the bulk chemical was stored in a sealed container under a nitrogen headspace, protected from light, at 2° to 8° C. No degradation of the bulk chemical was detected.

Acetone

ACS-grade acetone was obtained from Fisher Scientific (Hampton, NH) in two lots (963514 and 982335) that were used as the vehicle in the 2-week dermal study. The study laboratory determined the identity using IR spectroscopy and the purity using GC by system C (Table G1). IR spectra were consistent with a literature spectrum (*Aldrich*, 1981). GC indicated a major peak; two impurities of 0.15% and 0.05% of the total peak area; several minor impurities, each less than 0.01% of the total peak area; and an overall purity greater than 99.7%.

Corn Oil

Corn oil in multiple lots was used as the vehicle during the 2-week and 40-week gavage studies. The study laboratory analyzed peroxide levels prior to use and monthly during the study using potentiometric titration; all peroxide concentrations were less than 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

For the 2-week dermal study, the dose formulations were prepared once by pipetting the appropriate amounts of allyl bromide and acetone into a volumetric flask and mixing thoroughly (Table G2). The dose formulations were stored in amber glass vials under a headspace of inert gas, protected from light, at 2° to 8° C for up to 35 days.

Prior to the 2-week dermal study, the analytical chemistry laboratory conducted stability studies on 1 mg/mL formulations of allyl bromide in acetone using GC by system D (Table G1). Formulations were stored in glass vials capped with Teflon®-lined septa, protected from light, at 25° and 5° C, and at simulated animal room conditions. Stability was confirmed for up to 35 days at 25° and 5° C and up to 3 hours at animal room conditions.

For the 2-week and 40-week gavage studies, the appropriate amounts of allyl bromide and corn oil were pipetted into a volumetric flask and mixed thoroughly (Table G2). Dose formulations were prepared once for the 2-week study and every 2 weeks during the 40-week studies. Dose formulations were stored in amber glass vials with Teflon®-lined septa and aluminum crimp caps under a headspace of inert gas, protected from light, at 2° to 8° C for up to 21 days, with the exception of formulations used between November 16, 1999, and December 20, 1999, which were stored for 27 days. Dose formulations prepared on December 7, 1999, stored at 2° to 8° C for 28 days, then at –20° C until analyzed on January 13, 2000, confirmed stability for up to 28 days.

A solubility study of allyl bromide in corn oil was conducted at the analytical chemistry laboratory using GC by a system similar to system A; the maximum solubility was 142.2 mg/mL. No homogeneity studies were conducted on dose formulations in corn oil as concentrations used in the 2-week study (0.75 to 12.0 mg/mL) and 40-week studies (0.05 to 0.80 mg/mL) were well below the maximum solubility.

For the 2-week and 40-week gavage studies, the analytical chemistry laboratory conducted stability studies on 0.37 mg/mL formulations of allyl bromide in corn oil using GC by system E (Table G1). Formulations were stored in amber glass vials capped with Teflon®-lined septa, protected from light, at 25° and 5° C, and at simulated animal room conditions. Stability was confirmed for up to 16 days at 25° C, up to 21 days at 5° C, and up to 3 hours at animal room conditions. Later, a second stability study was conducted by the analytical chemistry laboratory on 0.74 mg/mL formulations under the same conditions as those previously described. No significant trend toward loss was observed at 25° or 5° C up to 42 days, though variability was large (RSD 10.8%), and no significant loss was observed at animal room conditions up to 3 hours.

Periodic analyses of the dose formulations were conducted by the study laboratory using GC by a system similar to system D (dermal) or a system similar to system E (gavage) . For the 2-week dermal and gavage studies, the dose formulations were analyzed once. Animal room samples were also analyzed. Of the dose formulations used and analyzed for the dermal study, all five were within 10% of the target concentrations; all five animal room samples were within 10% of the target concentrations (Table G4). Of the dose formulations used and analyzed for the 2-week gavage study, all five were within 10% of the target concentrations; one of five animal room samples was within 10% of the target concentrations (Table G5). For the 40-week gavage studies, formulations were analyzed at least every 12 weeks; animal room samples were also analyzed. Of the dose formulations used and analyzed, all 25 were within 10% of the target concentrations; 17 of 30 animal room samples were within 10% of the target concentrations (Table G3). Increased awareness of volatility issues and subsequent careful handling improved animal room sample results over the course of the 40-week studies.

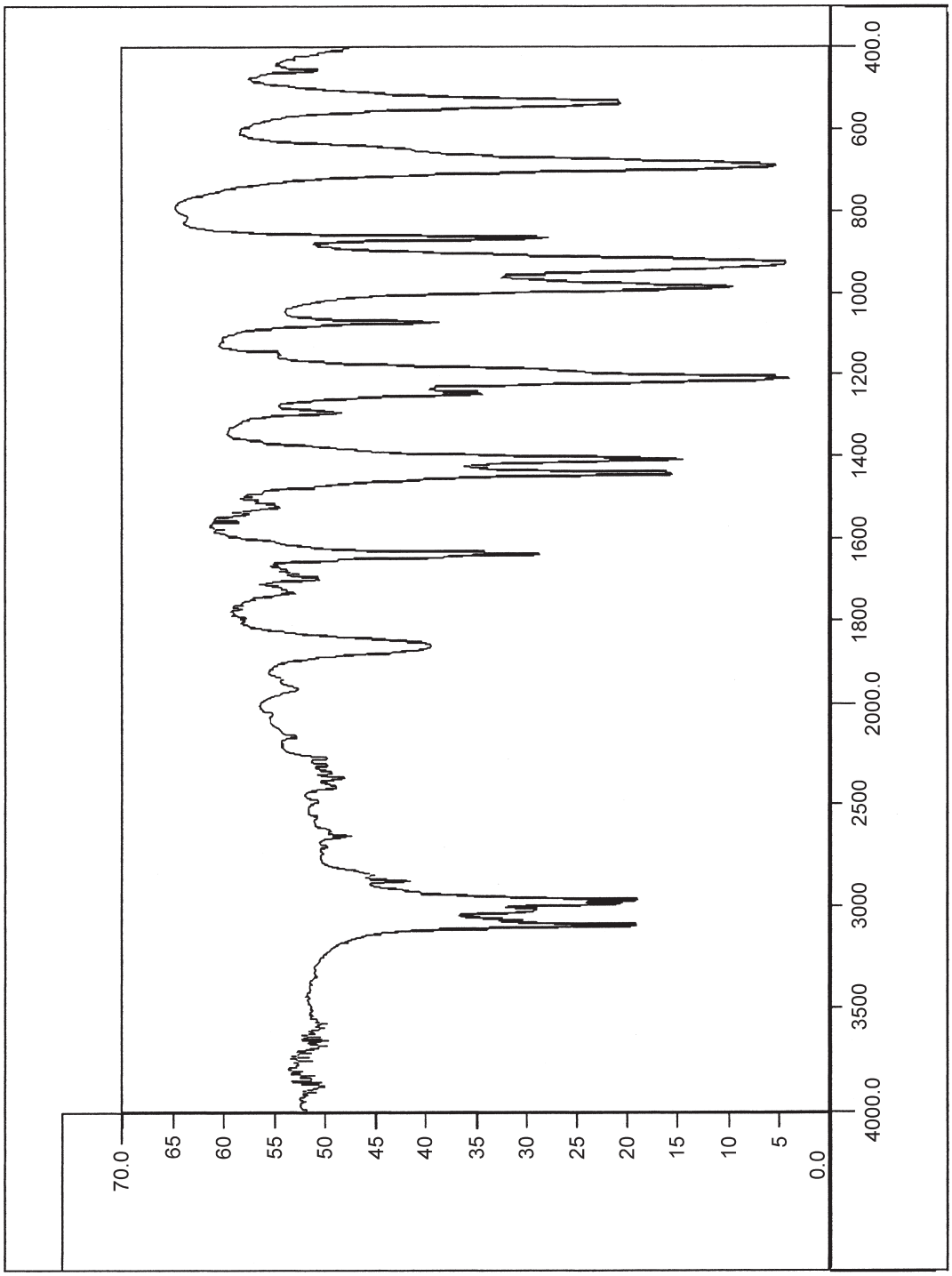

FIGURE G1
Infrared Absorption Spectrum of Allyl Bromide

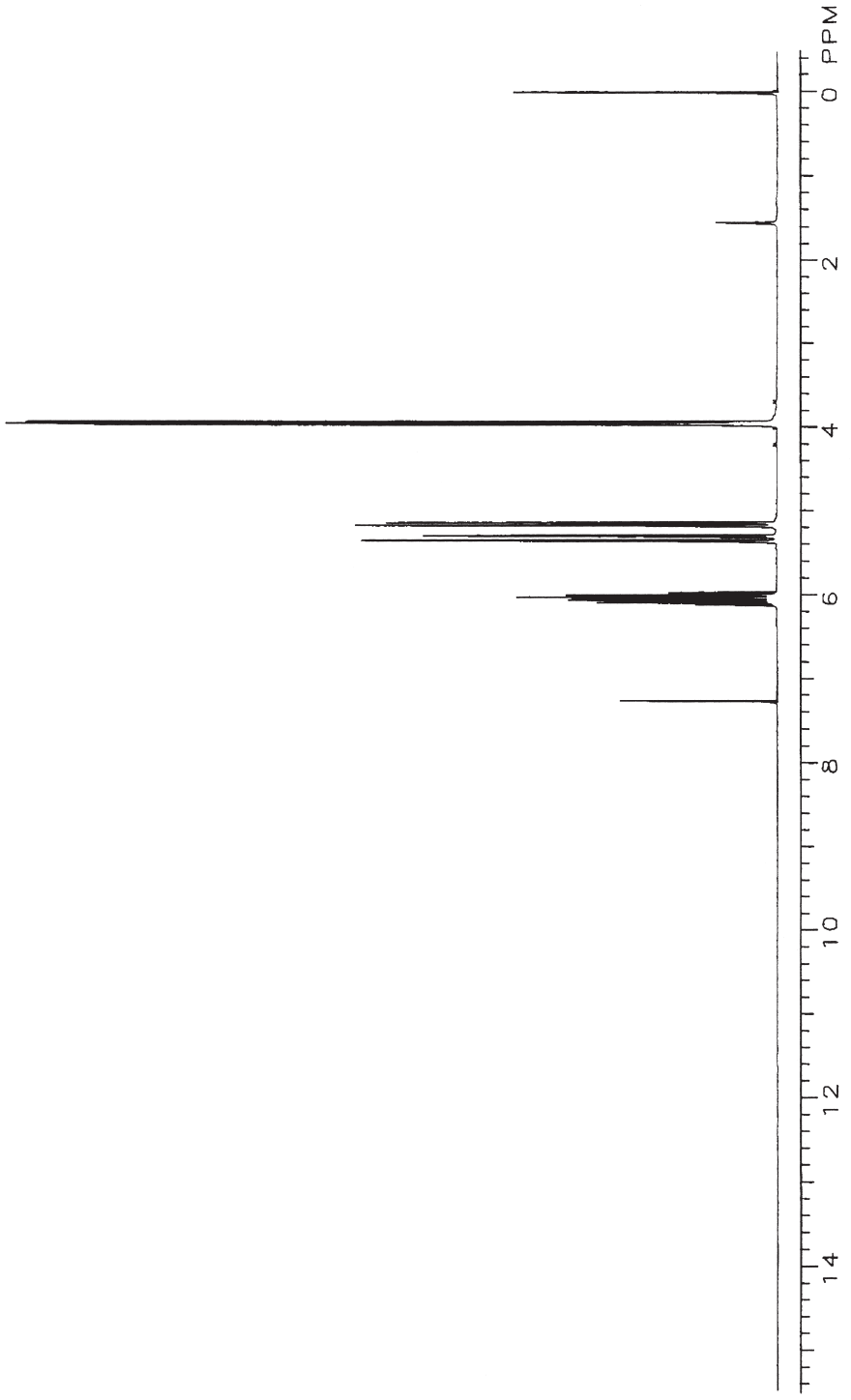

Figure G2
Proton Nuclear Magnetic Resonance Spectrum of Allyl Bromide

TABLE G1
Gas Chromatography Systems Used in the Studies of Allyl Bromide[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	DB-1, 30 m × 0.53 mm, 1.5-µm film thickness, (J&W Scientific, Folsom, CA)	Helium at 10 mL/minute	40° C held for 5 minutes, then to 250° C at 10° C/minute, held for 4 minutes
System B			
Flame ionization	DB-1, 30 m × 0.53 mm, 1.5-µm film thickness, (J&W Scientific)	Nitrogen at 20-23.3 mL/minute	40° C held for 5 minutes, then to 90° C at 10° C/minute
System C			
Flame ionization	DB-1, 30 m × 0.53 mm, 3-µm film thickness (J&W Scientific)	Nitrogen at 17.5 mL/minute and 25 psi	40° C held 4 minutes, then 170° C at 10° C/minute, held 1 minute
System D			
Electron capture	Rtx-1, 30 m × 0.53 mm, 3-µm film thickness (Restek, Bellafonte, PA)		40° C held 14 minutes, then 160° C at 40° C/minute, held 3 minutes
System E			
Flame ionization	Rtx-1, 30 m × 0.53 mm, 3-µm film thickness (Restek)	Helium at 10 mL/minute	40° C held 5 minutes, then 250° C at 50° C/minute, held 5 minutes

[a] Gas chromatographs were manufactured by Varian (Palo Alto, CA) (Systems A, D, and E) or Hewlett Packard (Palo Alto, CA) (Systems B and C)

TABLE G2
Preparation and Storage of Dose Formulations in the Dermal and Gavage Studies of Allyl Bromide

Dermal Study	Gavage Studies
Preparation	
The required amounts of allyl bromide and ACS-grade acetone were pipetted into a volumetric flask and mixed thoroughly. Dose formulations were prepared once.	The required amount of allyl bromide was added to corn oil in a volumetric flask and mixed thoroughly. Dose formulations were prepared once every 2 weeks.
Chemical Lot Number	
330638	03614HN and 330638
Maximum Storage Time	
35 days	21 days[a]
Storage Conditions	
Formulations were transferred to 5-mL amber glass vials, sealed under a nitrogen headspace, and refrigerated at 2° to 8° C.	Stored in sealed amber glass vials under a headspace of inert gas, protected from light, at 2° to 8° C.
Study Laboratory	
BioReliance (Rockville, MD)	BioReliance (Rockville, MD)

[a] Dose formulations used between November 16, 1999, and December 20, 1999, were stored for 27 days. A subsequent stability study on formulations prepared December 7, 1999, stored at 2° to 8° C for 28 days, then at –20° C until analyzed, confirmed stability for 28 days; all formulations were within 10% of target concentrations (Table G3).

TABLE G3
Results of Analyses of Dose Formulations Administered to FVB/N, C57BL/6, Tg.AC Hemizygous, and p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
March 16, 1999	March 16, 1999	0.05	0.0455	–9
		0.10	0.0953	–5
		0.20	0.188	–6
		0.40	0.415	+4
		0.80	0.826	+3
	April 14, 1999[b]	0.05	0.0332	–34
		0.10	0.0541	–46
		0.20	0.103	–49
		0.40	0.286	–29
		0.80	0.607	–24
May 11, 1999	May 11, 1999	0.05	0.0475	–5
		0.10	0.0959	–4
		0.20	0.187	–7
		0.40	0.413	+3
		0.80	0.756	–6
	June 3, 1999[b]	0.05	0.0289	–42
		0.10	0.0489	–51
		0.20	0.116	–42
		0.40	0.193	–52
		0.80	0.354	–56
August 3, 1999	August 3, 1999	0.05	0.0462	–8
		0.10	0.0920	–8
		0.20	0.183	–9
		0.40	0.374	–7
		0.80	0.741	–7
	August 24, 1999[b]	0.05	0.0450	–10
		0.10	0.0692	–31
		0.20	0.196	–2
		0.40	0.304	–24
		0.80	0.784	–2

TABLE G3
Results of Analyses of Dose Formulations Administered to FVB/N, C57BL/6, Tg.AC Hemizygous, and p53 Haploinsufficient Mice in the 40-Week Gavage Study of Allyl Bromide

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
September 28, 1999	September 28, 1999	0.05	0.0525	+5
		0.10	0.100	0
		0.20	0.190	–5
		0.40	0.379	–5
		0.80	0.731	–9
	October 22, 1999[b]	0.05	0.0476	–5
		0.10	0.0912	–9
		0.20	0.198	–1
		0.40	0.359	–10
		0.80	0.775	–3
December 7, 1999	January 13, 2000[c]	0.05	0.0527	+5
		0.10	0.0916	–8
		0.20	0.187	–7
		0.40	0.407	+2
		0.80	0.763	–5
	January 13, 2000[b]	0.05	0.0481	–4
		0.10	0.0733	–27
		0.20	0.195	–3
		0.40	0.400	0
		0.80	0.759	–5
December 21, 1999	December 21, 1999	0.05	0.0550	+10
		0.10	0.109	+9
		0.20	0.218	+9
		0.40	0.437	+9
		0.80	0.854	+7
	January 13, 2000[b]	0.05	0.0548	+10
		0.10	0.108	+8
		0.20	0.198	–1
		0.40	0.404	+1
		0.80	0.814	+2

[a] Results of duplicate analyses. Dosing volume=10 mL/kg; 0.05 mg/mL=0.5 mg/kg, 0.10 mg/mL=1.0 mg/kg, 0.20 mg/mL=2 mg/kg, 0.40 mg/mL=4 mg/kg, 0.80 mg/mL=8 mg/kg
[b] Animal room samples
[c] Used in study but not analyzed at the time of preparation; samples refrigerated until analysis

TABLE G4
Results of Analyses of Dose Formulations Administered to FVB/N Mice in the 2-Week Dermal Study of Allyl Bromide

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
September 9, 1998	September 10, 1998	2.27	2.39	+5
		4.55	4.73	+4
		9.09	8.96	–1
		18.2	18.40	+1
		36.4	36.90	+1
	October 2, 1998[b]	2.27	2.13	–6
		4.55	4.39	–3
		9.09	8.64	–5
		18.2	17.9	–2
		36.4	34.5	–5

[a] Results of duplicate analyses. Dosing volume=3.3 mL/kg; 2.27 mg/mL=7.5 mg/kg, 4.55 mg/mL=15 mg/kg, 9.09 mg/mL=30 mg/kg, 18.2 mg/mL=60 mg/kg, 36.4 mg/mL=120 mg/kg
[b] Animal room samples

TABLE G5
Results of Analyses of Dose Formulations Administered to C57BL/6 Mice in the 2-Week Gavage Study of Allyl Bromide

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
September 10, 1998	September 11, 1998	0.75	0.77	+3
		1.5	1.56	+4
		3.0	3.02	+1
		6.0	6.09	+2
		12.0	12.8	+7
	October 5, 1998[b]	0.75	0.493	–34
		1.5	1.05	–30
		3.0	2.52	–16
		6.0	5.02	–16
		12.0	11.4	–5

[a] Results of duplicate analyses. Dosing volume=10 mL/kg; 0.75 mg/mL=7.5 mg/kg, 1.5 mg/mL=15 mg/kg, 3.0 mg/mL=30 mg/kg, 6.0 mg/mL=60 mg/kg, 12.0 mg/mL=120 mg/kg
[b] Animal room samples