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

NTP Technical Report on the Toxicology and Carcinogenesis Studies of Propargyl Alcohol (CAS No. 107-19-7) in F344/N Rats and B6C3F1 Mice (Inhalation Studies)

NTP TR 552

SEPTEMBER 2008

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF PROPARGYL ALCOHOL

(CAS NO. 107-19-7)

IN F344/N RATS AND B6C3F1 MICE

(INHALATION STUDIES)

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

September 2008

NTP 552

NIH Publication No. 08-5893

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 Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). 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 Technical 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.

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CONTENTS

SUMMARY

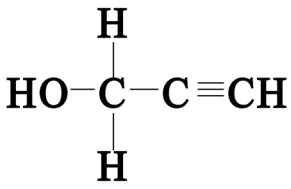
Background
Propargyl alcohol is used in a variety of chemical manufacturing processes and to protect steel from becoming brittle. We studied propargyl alcohol to determine if it caused cancer in rats or mice.

Methods
We exposed groups of 50 male and female rats and mice to air containing propargyl alcohol six hours per day for two years. Rats were exposed to concentrations of 16, 32, or 64 parts per million (ppm) of propargyl alcohol in air, and mice were exposed to concentrations of 8, 16, or 32 ppm. Similar groups of 50 animals were exposed to clean air in the same inhalation chambers six hours per day as the untreated control groups. Tissues from more than 40 sites were examined for every animal.

Results
All groups of animals exposed to propargyl alcohol experienced a variety of lesions in the epithelial tissues of the nose, including hyperplasia, metaplasia, and inflammation. Adenomas of the respiratory epithelium of the nose were observed in male rats and in male and female mice exposed to propargyl alcohol. The rate of mononuclear cell leukemia was also increased in exposed male rats, and the rate of Harderian gland adenomas was increased in exposed male mice.

Conclusions
We conclude that the increased occurrences of adenomas of the epithelium of the nose in male rats and in male and female mice and of mononuclear cell leukemia in male rats were caused by exposure to propargyl alcohol. An increased occurrence of adenomas of the Harderian gland in male mice may also have been associated with exposure to propargyl alcohol.

ABSTRACT



Propargyl Alcohol

CAS No. 107-19-7

Chemical Formula: C_3H_4O Molecular Weight: 56.06

Synonyms: Ethynylcarbinol; 1-hydroxy-2-propyne; 3-hydroxy-1-propyne; PA; 1-propyn-3-ol; 1-propyn-3-yl alcohol; 2-propynol; 3-propynol; propynyl alcohol; 2-propynyl alcohol

Propargyl alcohol is a commercially available acetylenic primary alcohol. It is also a by-product in the industrial synthesis of butynediol from acetylene and formaldehyde with copper acetylide as catalyst. Propargyl alcohol is used as a reactant/chemical intermediate, pharmaceutical intermediate, agricultural chemical intermediate, soil fumigant, corrosion inhibitor, solvent stabilizer, and polymer modifier. It has also been used to prevent the hydrogen embrittlement of steel. Propargyl alcohol was nominated by the National Cancer Institute for study because of the potential for human exposure in occupational settings through inhalation and dermal contact. Male and female F344/N rats and B6C3F1 mice were exposed to propargyl alcohol (greater than 99% pure) by inhalation for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and mouse peripheral blood erythrocytes.

2-Week Study in Rats

Groups of five male and five female rats were exposed to propargyl alcohol vapor at concentrations of 0, 31.3, 62.5, 125, 250, or 500 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 16 days. All males exposed to 125 ppm or greater and all females exposed to 250 or 500 ppm died by the end of day 3 of the study, and one 125 ppm female died on day 5. Mean body weights were significantly decreased in 62.5 ppm males and 125 ppm females. Clinical findings in the 125 and 250 ppm groups included lethargy, ataxia, abnormal breathing, and nasal/eye discharge. Right kidney weights of 62.5 and 125 ppm females and liver weights of 125 ppm females were significantly greater than those of the chamber controls. All 250 and 500 ppm males and females had moderate to marked periportal necrosis, congestion, and erythrophagocytosis of the liver.

2-Week Study in Mice

Groups of five male and five female mice were exposed to propargyl alcohol vapor at concentrations of 0, 31.3, 62.5, 125, 250, or 500 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 17 days. All mice exposed to 125 ppm or greater died by day 3 of the study. Mean body weights of mice exposed to 62.5 ppm were significantly less than those of the chamber controls. Clinical findings in the 62.5 and/or 125 ppm groups included abnormal breathing, nasal/eye discharge, thinness, and lethargy. Right kidney weights of 31.3 ppm mice were significantly greater, and thymus weights of 62.5 ppm males were significantly less than those of the chamber controls. The livers of all males and females exposed to

250 or 500 ppm exhibited marked periportal necrosis, congestion, and erythro-phagocytosis; these lesions also occurred in all 125 ppm males with less severity.

3-Month Study in Rats

Groups of 10 male and 10 female rats were exposed to propargyl alcohol vapor at concentrations of 0, 4, 8, 16, 32, or 64 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. All rats survived to the end of the study. Mean body weights of all exposed groups were similar to those of the chamber control groups. The incidences of minimal to mild hyperplasia of respiratory epithelium of the nose were significantly increased in all exposed groups except 8 ppm males and 4 ppm females. Squamous metaplasia of the respiratory epithelium was noted in a few males and most females exposed to 64 ppm. Necrosis of olfactory epithelium was present in half of the males and females exposed to 64 ppm and in a few males and females exposed to 32 ppm.

3-Month Study in Mice

Groups of 10 male and 10 female mice were exposed to propargyl alcohol vapor at concentrations of 0, 4, 8, 16, 32, or 64 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. All mice survived to the end of the study. Mean body weights of males exposed to 8 ppm or greater and 32 and 64 ppm females were significantly less than those of the chamber control groups. Histopathologic changes occurred in the nasal cavity of mice and involved both the respiratory and olfactory epithelium in groups exposed to 16 ppm or greater. Lesions included minimal to moderate suppurative inflammation, minimal to moderate squamous metaplasia of the respiratory epithelium, minimal to mild hyaline degeneration (accumulation) in the respiratory epithelium, minimal to moderate olfactory epithelial atrophy, minimal to moderate hyperplasia of glands in the olfactory region, minimal necrosis of olfactory epithelium, and minimal to moderate turbinate atrophy.

There were no biologically significant differences in organ weights between exposed and chamber control groups. Reproductive tissue parameters of exposed males were similar to those of the chamber controls. Only 2/9 female mice in the 64 ppm group exhibited regular estrous cyclicity compared to 6/10 in the controls. Females exposed to 16 ppm differed from chamber controls in the relative time in the estrous stages, and 64 ppm females had a significantly increased probability of extended estrus. No gross lesions were observed at necropsy.

2-Year Study in Rats

Groups of 50 male and 50 female rats were exposed to propargyl alcohol vapor at concentrations of 0, 16, 32, or 64 ppm, 6 hours plus T_{90} (14 minutes) per day, 5 days per week for 105 weeks. Survival of 32 and 64 ppm males was significantly less than that of the chamber control group. Mean body weights of males exposed to 64 ppm were less than those of the chamber controls after week 24 of the study.

Nasal respiratory epithelial adenomas were present in three 64 ppm males and one 32 ppm female; the incidence in 64 ppm males exceeded the historical control ranges. A spectrum of nonneoplastic lesions occurred in the respiratory and olfactory epithelium of rats at all exposure concentrations. The incidences of respiratory epithelial hyperplasia, respiratory glandular hyperplasia, and olfactory basal cell hyperplasia were significantly increased in all exposed groups of rats. The incidences of lesions of the olfactory epithelium including hyperplasia, glandular hyperplasia, atrophy, respiratory metaplasia, degeneration, necrosis, hyaline droplet accumulation, and chronic active inflammation were significantly increased in one or more exposed groups of males and/or females.

The incidence of mononuclear cell leukemia was significantly increased in males exposed to 64 ppm, and the incidence exceeded the historical control ranges.

2-Year Study in Mice

Groups of 50 male and 50 female mice were exposed to propargyl alcohol vapor at concentrations of 0, 8, 16, or 32 ppm, 6 hours plus T_{90} (14 minutes) per day, 5 days per week for 105 weeks. Survival of exposed groups was similar to that of the chamber control groups. Mean body weights of 16 and 32 ppm females were less than those of the chamber control group after weeks 73 and 21, respectively. Eye abnormality (unspecified) was

observed after one full year of exposure with the incidence increasing in an exposure concentration-related manner.

The incidences of nasal respiratory epithelial adenoma increased with a positive trend and were significantly increased in groups exposed to 32 ppm. A spectrum of nonneoplastic lesions occurred in the nasal respiratory and olfactory epithelium of mice at all exposure concentrations. The incidences of respiratory epithelial hyperplasia, respiratory glandular hyperplasia, and squamous metaplasia were significantly increased in most exposed groups of mice. Suppurative inflammation was often associated with the squamous metaplasia, and turbinate atrophy was present in all exposed mice (except one 16 ppm male). The incidences of olfactory epithelial atrophy and respiratory metaplasia were increased in the 16 and 32 ppm groups. Significantly increased incidences of Harderian gland adenoma occurred in 8 and 32 ppm males.

Genetic Toxicology

Propargyl alcohol was mutagenic in *Salmonella typhimurium* strain TA100 in the absence of liver S9 activation enzymes only; no mutagenicity was observed in TA100 in the presence of S9 enzymes, in TA1535 without S9, or in TA98 with or without S9. *In vivo*, no significant increases in the frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood samples from male mice exposed by inhalation to propargyl alcohol for 3 months. In female mice, propargyl alcohol exposure produced a small increase in micronucleated erythrocytes that was judged to be equivocal. No significant changes in the percentage of polychromatic erythrocytes were seen in either male or female mice after 3 months of exposure to propargyl alcohol.

Conclusions

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity** of propargyl alcohol in male F344/N rats based on increased incidences of nasal respiratory epithelial adenoma and mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of propargyl alcohol in female F344/N rats exposed to 16, 32, or 64 ppm. There was *some evidence of carcinogenic activity* of propargyl alcohol in male and female B6C3F1 mice based on increased incidences of nasal respiratory epithelial adenoma. The increased incidences of Harderian gland adenoma in male B6C3F1 mice may have been related to exposure to propargyl alcohol.

Exposure to propargyl alcohol resulted in increased incidences of nonneoplastic nasal lesions in male and female rats and mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Propargyl Alcohol

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Concentrations in air	0, 16, 32, or 64 ppm	0, 16, 32, or 64 ppm	0, 8, 16, or 32 ppm	0, 8, 16, or 32 ppm
Body weights	64 ppm group less than the chamber control group	Exposed groups similar to the chamber control group	Exposed groups similar to the chamber control group	16 and 32 ppm groups less than the chamber control group
Survival rates	27/50, 23/50, 15/50, 16/50	29/50, 31/50, 27/50, 26/50	37/50, 43/50, 40/50, 42/50	39/50, 39/50, 32/50, 38/50
Nonneoplastic effects	Nose: hyperplasia, respiratory epithelium (5/49, 21/49, 44/50, 42/49); hyperplasia, glands, respiratory epithelium (3/49, 14/49, 39/50, 45/49); hyperplasia, basal cell, olfactory epithelium (0/49, 19/49, 42/50, 42/49); hyperplasia, olfactory epithelium (0/49, 1/49, 3/50, 5/49); hyperplasia, glands, olfactory epithelium (0/49, 0/49, 0/50, 4/49); atrophy, olfactory epithelium (1/49, 21/49, 26/50, 26/49); metaplasia, respiratory, olfactory epithelium (1/49, 10/49, 18/50, 29/49); degeneration, olfactory epithelium (0/49, 0/49, 1/50, 7/49); necrosis, olfactory epithelium (0/49, 0/49, 2/50, 6/49); hyaline droplet accumulation, olfactory epithelium (0/49, 5/49, 4/50, 7/49); inflammation, chronic active (9/49, 12/49, 22/50, 28/49)	Nose: hyperplasia, respiratory epithelium (2/49, 23/49, 25/50, 36/50); hyperplasia, glands, respiratory epithelium (2/49, 33/49, 44/50, 47/50); hyperplasia, basal cell, olfactory epithelium (0/49, 28/49, 42/50, 48/50); hyperplasia, glands, olfactory epithelium (0/49, 6/49, 1/50, 2/50); atrophy, olfactory epithelium (3/49, 0/49, 28/50, 37/50); metaplasia, respiratory, olfactory epithelium (3/49, 2/49, 7/50, 17/50); necrosis, olfactory epithelium (0/49, 0/49, 2/50, 5/50); hyaline droplet accumulation, olfactory epithelium, (6/49, 5/49, 6/50, 15/50); inflammation, chronic active (7/49, 9/49, 11/50, 18/50)	Nose: hyperplasia, respiratory epithelium (1/49, 49/50, 49/50, 50/50); hyperplasia, glands, respiratory epithelium (17/49, 29/50, 40/50, 50/50); metaplasia, squamous, respiratory epithelium (2/49, 11/50, 36/50, 50/50); inflammation, suppurative (2/49, 16/50, 25/50, 50/50); atrophy, turbinate (0/49, 50/50, 49/50, 50/50); atrophy, olfactory epithelium (0/49, 3/50, 21/50, 33/50); metaplasia, respiratory, olfactory epithelium (5/49, 0/50, 7/50, 16/50)	Nose: hyperplasia, respiratory epithelium (0/50, 50/50, 50/50, 49/50); hyperplasia, glands, respiratory epithelium (7/50, 24/50, 44/50, 49/50); metaplasia, squamous, respiratory epithelium (0/50, 3/50, 34/50, 49/50); inflammation, suppurative (1/50, 4/50, 35/50, 45/50); atrophy, turbinate (0/50, 50/50, 50/50, 50/50); atrophy, olfactory epithelium (2/50, 5/50, 31/50, 29/50); metaplasia, respiratory, olfactory epithelium (1/50, 0/50, 6/50, 14/50)
		None		
Neoplastic effects	Nose: adenoma, respiratory epithelium (0/49, 0/49, 0/50, 3/49)		Nose: adenoma, respiratory epithelium (0/49, 1/50 , 4/50, 7/50)	Nose: adenoma, respiratory epithelium (0/50, 2/50, 4/50, 6/50)
	Mononuclear cell leukemia: (21/49, 26/50, 23/50, 37/50)			
		None		
Equivocal findings	None		Harderian gland: adenoma (3/50, 10/50, 6/50, 11/50)	None
		No evidence		
Level of evidence of carcinogenic activity	Some evidence		Some evidence	Some evidence

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Propargyl Alcohol

Genetic toxicology	
Salmonella typhimurium gene mutations:	Positive in strain TA100 without S9, negative with S9; negative in strain TA98, with and without S9; negative in strain TA1535 without S9
Micronucleated erythrocytes	
Mouse peripheral blood *in vivo:*	Negative in males; equivocal in 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 Technical Report on propargyl alcohol on May 17, 2007, 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 May 17, 2007, the draft Technical Report on the toxicology and carcinogenesis studies of propargyl alcohol 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. M.J. Hooth, NIEHS, described the toxicology and carcinogenesis studies of propargyl alcohol by describing the uses of the chemical, the survival and body weights in the short-term studies, and the neoplastic and nonneoplastic lesions observed in the 2-year studies. The proposed conclusions for the 2-year inhalation study in rats were *some evidence of carcinogenic activity* of propargyl alcohol in male F344/N rats, *no evidence of carcinogenic activity* of propargyl alcohol in female F344/N rats, and *clear evidence of carcinogenic activity* of propargyl alcohol in male and female B6C3F1 mice.

Dr. Bradfield, the first principal reviewer, inquired about the criteria for formulating level of evidence conclusions and particularly about the interpretation of benign tumors such as adenomas. Dr. D.E. Malarkey, NIEHS, replied there was some suggestion from a couple of studies that nasal respiratory adenomas may have the potential to progress to malignancy.

Dr. Crump, the second principal reviewer, questioned whether the effects in the mouse nasal epithelium, though occurring in both sexes, rose to the level of clear evidence in the absence of any malignant tumors.

Dr. Cattley, the third principal reviewer, also questioned whether the appropriate conclusion for mice was clear evidence based on the given criteria. He felt the question was whether the ability of the adenomas to progress to malignancy was well established and questioned what amount of increase was required to be considered "marked."

Dr. Bradfield asked if more could be said about the mechanism of tumor induction. Dr. G.P. Flake, NIEHS, replied that the cytochrome P450s are more concentrated in the olfactory epithelium and also localized in the transitional epithelium of the respiratory mucosa, the areas that develop adenomas. Dr. Novak added that the turnover of nasal epithelial cells was quite rapid.

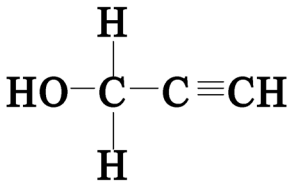
In discussion of the levels of evidence for the mouse studies, Dr. Walker noted that the nasal tumors occurred in both sexes. Dr. Malarkey said that these were rare tumors, and thus the number of opportunities to observe progression to malignancy was also small. He added for comparison that in dogs, nasal adenomas, though considered benign, would continue to grow and act malignant, destroying the nasal tissue and invading the brain. Dr. Malarkey felt that for a tumor with a background incidence of 0.1%, an occurrence of 20% in an exposed group could be considered a marked increase. Dr. Cattley said that in this study there were nearly 100% incidences of hyperplasia of the respiratory epithelium and glandular respiratory epithelium, squamous metaplasia of the respiratory epithelium, and suppurative inflammation of the nasal passages, which would provide a severe test of the ability of these lesions to progress.

Dr. Pino asked for more explanation of why the mononuclear cell leukemia in male rats was considered some evidence. Dr. Walker explained that the effect was seen in just one exposed group, and for a commonly occurring tumor, the trend test may be more relevant than one individual pairwise comparison.

Dr. Cattley said that no malignant nasal neoplasms were seen in mice with many nonneoplastic lesions, and Dr. Crump noted that opinion among pathologists seemed divided on whether there is evidence that a particular type of adenoma can progress to malignancy. Dr. Flake said the WHO International Classification of Tumors states that nasal adenomas can arise from respiratory epithelium, mucosal glands, or from preexisting adenoma. Two literature sources indicated that these adenomas and carcinomas form a continuum with possible progression, but not with a high frequency.

Dr. Soper moved, and Dr. Crump seconded, that the conclusions be accepted as written but with the conclusion in male mice changed from clear evidence to some evidence. The motion was approved by four votes to two (Drs. Deininger and Walker).

INTRODUCTION



Propargyl Alcohol

CAS No. 107-19-7

Chemical Formula: C_3H_4O Molecular Weight: 56.06

Synonyms: Ethynylcarbinol; 1-hydroxy-2-propyne; 3-hydroxy-1-propyne; PA; 1-propyn-3-ol; 1-propyn-3-yl alcohol; 2-propynol; 3-propynol; propynyl alcohol; 2-propynyl alcohol

Chemical and Physical Properties

Propargyl alcohol is a moderately volatile, clear to slightly straw-colored liquid with a mild geranium-like odor. It has a melting point of –52° C, a boiling point of 114° C, and a vapor pressure of 11.6 mm Hg at 20° C. It is soluble in water, ethanol, benzene, acetone, chloroform, and ether and is insoluble in aliphatic hydrocarbons (Lington and Bevan, 1994; ACGIH, 2005).

Production, Use, and Human Exposure

Propargyl alcohol is a commercially available acetylenic primary alcohol that can be prepared from acetylene by high-pressure synthesis (Lewis, 1993). It is also a by-product in the industrial synthesis of butynediol from acetylene and formaldehyde with copper acetylide as catalyst. In the usual high-pressure butynediol process, about 5% of the product is propargyl alcohol. However, the reaction can be designed to obtain more propargyl alcohol using high acetylene pressure and low formaldehyde concentration (Lington and Bevan, 1994; BUA, 1998). Propargyl alcohol can also be prepared by heating beta-bromoallyl alcohol with concentrated potassium hydroxide (*Merck*, 1989).

Propargyl alcohol is listed in the Environmental Protection Agency's Toxic Substances Control Act Inventory (HSDB, 2005). The estimated annual production of propargyl alcohol in the United States was reported to range from 480,000 to 2,770,000 pounds (TRI, 1996). The sole manufacturer of propargyl alcohol in Europe (BASF AG; Ludwigshafen, Germany) produced from 500 to 1,000 tons/year in 1996 and 1997 with exports worldwide (BUA, 1998). Quantities of propargyl alcohol reported to have been imported from Europe to the United States exceeded 60,000 pounds between January and August, 1995, and 171,000 pounds between March and December, 1994 (DIS, 1995).

Propargyl alcohol is used as a reactant/chemical intermediate, pharmaceutical intermediate, agricultural chemical intermediate, soil fumigant, corrosion inhibitor, solvent stabilizer, and polymer modifier. It has also been used to prevent the hydrogen embrittlement of steel (Lewis, 1993; Kuney, 1994; Lington and Bevan, 1994; ACGIH, 2005).

There is potential for human exposure to propargyl alcohol in occupational settings through inhalation and dermal contact (Lington and Bevan, 1994). The National

Occupational Exposure Survey estimated that 54,358 workers were potentially exposed to propargyl alcohol in the workplace (NIOSH, 1990). Propargyl alcohol is not known to occur naturally but has been identified as a pollutant in air, soil, and solid waste.

The ACGIH-recommended threshold limit value time-weighted average (TLV-TWA) for propargyl alcohol is 1 ppm (2.3 mg/m^3) with a recommended skin notation because of its rapid skin absorption. This TLV is based on structural and toxicological similarities to allyl alcohol, which has a TLV-TWA of 2 ppm (ACGIH, 2005). The OSHA permissible exposure limit (PEL) is 1 ppm (2.3 mg/m^3) over an 8-hour work shift with a skin notation.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

Absorption, distribution, metabolism, and excretion studies in male F344 rats and B6C3F1 mice following exposure by intravenous, oral, dermal, or inhalation routes have been reported (Dix *et al*., 2001). Dermal absorption was minimal because of the volatility of the chemical. Comparing disposition following intravenous and oral exposure led to the estimate of 85% to 90% absorption of a 50 mg propargyl alcohol/kg body weight oral dose by each species. Absorption from inhalation exposure concentrations of 1, 10, and 100 ppm was approximately 60% for the 1 and 10 ppm exposure concentrations, but only 20% to 30% of the highest exposure concentration was absorbed. The highest concentrations of [^{14}C]-propargyl alcohol-derived radioactivity were in liver and kidney following intravenous administration. The radioactivity was cleared from the tissues rapidly. In mice, there was an 18-fold decrease in concentration of radioactivity in liver and a 6-fold decrease in kidney between 1 and 24 hours. In the same time span in rats, the decrease was 12-fold in liver and 6-fold in kidney. The three major excretion routes were urine, feces, and exhalation of $^{14}CO_2$, accounting for 62%, 6%, and 19% of the administered dose in rats and 40%, 10%, and 26% in mice, respectively. The more rapid clearance of [^{14}C]-propargyl alcohol derived radioactivity from tissues is not reflected in the excretion data. Intravenous administration of [^{14}C]-propargyl alcohol to bile-duct-cannulated rats resulted in excretion of 60% of the administered dose in bile within 4 hours after administration. Less than 10% of the administered dose was excreted in feces in intact rats during 72 hours indicating that metabolites formed in liver and excreted in bile were further metabolized in the gut and reabsorbed.

The metabolism of propargyl alcohol to propargylaldehyde is not catalyzed by alcohol dehydrogenase but is oxidized by catalase (DeMaster *et al*., 1994). Metabolism of propargyl alcohol by cytochrome P450 CYP2E1 has also been reported (Moridani *et al*., 2001). Propargylaldehyde is a reactive chemical, especially as a Michael acceptor. *In vivo* metabolism of propargyl alcohol has been investigated using [1,2,3-^{14}C]-propargyl alcohol and ^{13}C nuclear magnetic resonance (Banijamali *et al*., 1999, 2000; Table 1; Figures 1 and 2). Virtually all identified metabolites are derived from oxidation of the alcohol function to an aldehyde that reacts once or twice with glutathione. Other metabolites result from the catabolism of the glutathione adducts to cysteine or *N*-acetylcysteine adducts and either oxidation of the aldehyde to the carboxylic acid or reduction back to the alcohol. These mercapturic pathway metabolites account for about 92% of the urinary radioactivity in mice. Rat metabolites R1 and R2 are not typical mercapturic acid pathway products. They account for 27% of the urinary radioactivity leaving only about 40% of the urinary metabolites as obvious mercapturic acid pathway products. The complexity of the urinary metabolite profile may be due to the metabolism by gut flora mentioned above.

Humans

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

TOXICITY

Experimental Animals

Propargyl alcohol is acutely toxic by the oral, dermal, and inhalation routes of exposure. Primary targets include the mucous membranes, liver, and kidney. It is irritating to the eyes, skin, and respiratory tract.

Reported acute oral LD_{50}s are 93 mg/kg (Vernot *et al*., 1977) and 110 mg/kg (Archer, 1985) for male Sprague-Dawley rats and 54 mg/kg (Vernot *et al*., 1977) and 55 mg/kg for female Sprague-Dawley rats (Archer, 1985). The acute oral LD_{50} is 50 mg/kg for mice and 60 mg/kg for guinea pigs (Rowe and McCollister,

TABLE 1
Quantitation of Urinary Metabolites of Propargyl Alcohol

	Identified Metabolite	Percentage of Urinary Radioactivity	
		Rat	Mouse
R1	3-(carboxymethylthio)-2-propenoic acid	20	0
R2	3-(methylsulfinyl)-2-(methylthio)-2-propenoic acid	7	0
R3, M5	3-[[2-(acetylamino)-2-carboxyethyl]thio]-3-[[2-(amino)-2-carboxyethyl]thio]-1-propanol	8	17
R4, M6	3-bis[[2-(acetylamino)-2-carboxyethyl]thio]-1-propanol	20	6
R5	3-[[2-(acetylamino)-2-carboxyethyl]thio]-3-[[2-(acetylamino)-2-carboxyethyl]sulfinyl]-1-propanol	15	0
M1	Propargyl glucuronide	0	6
M2	3-[(2-amino-2-carboxyethyl)thio]-2-propenoic acid (E and Z isomers)	0	41
M3	3-[(2-formylamino-2-carboxyethyl)thio]-2-propenoic acid	0	13
M4	3,3-bis[[2-(amino)-2-carboxyethyl]thio]-1-propanol	0	15
Total		70	98

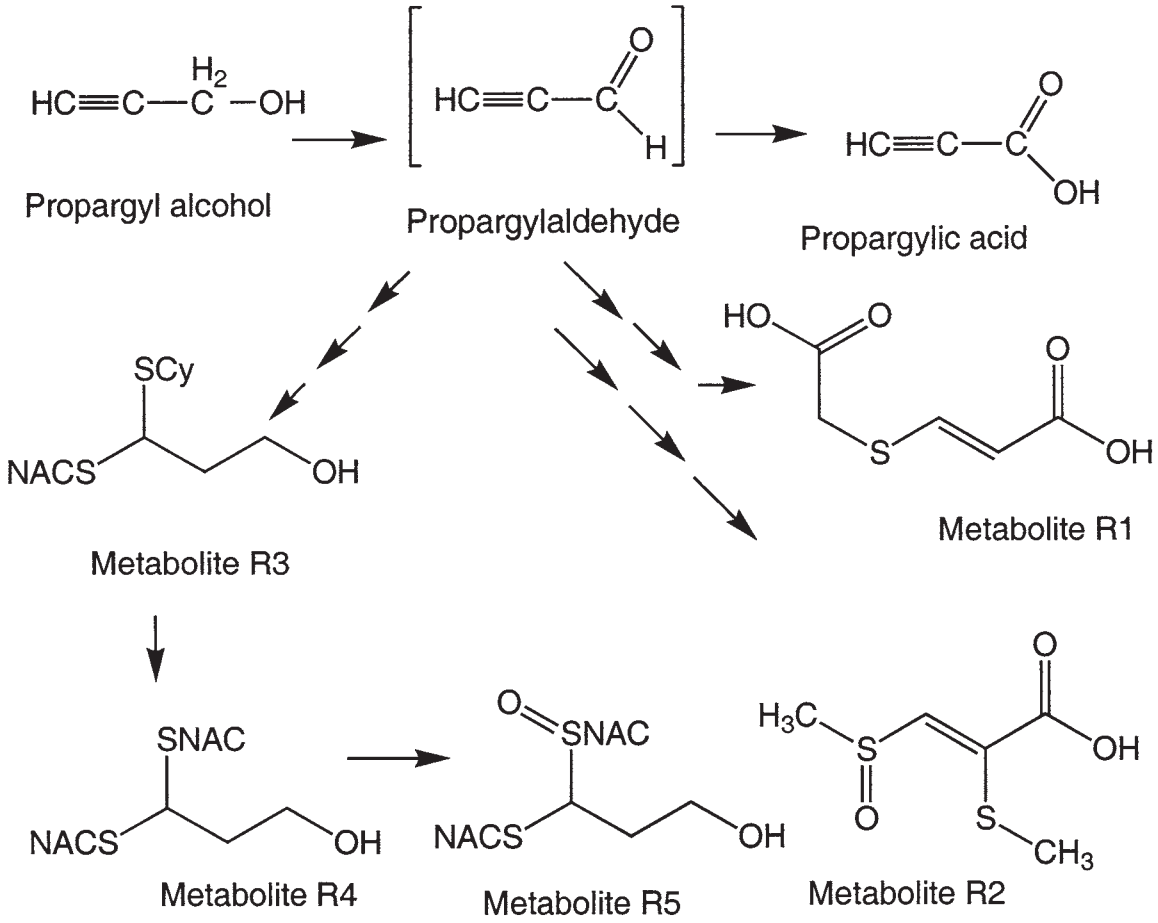


FIGURE 1
Metabolism of Propargyl Alcohol by Rats
SNAC, NACS=*N*-acetylcysteine; SCy=cysteine

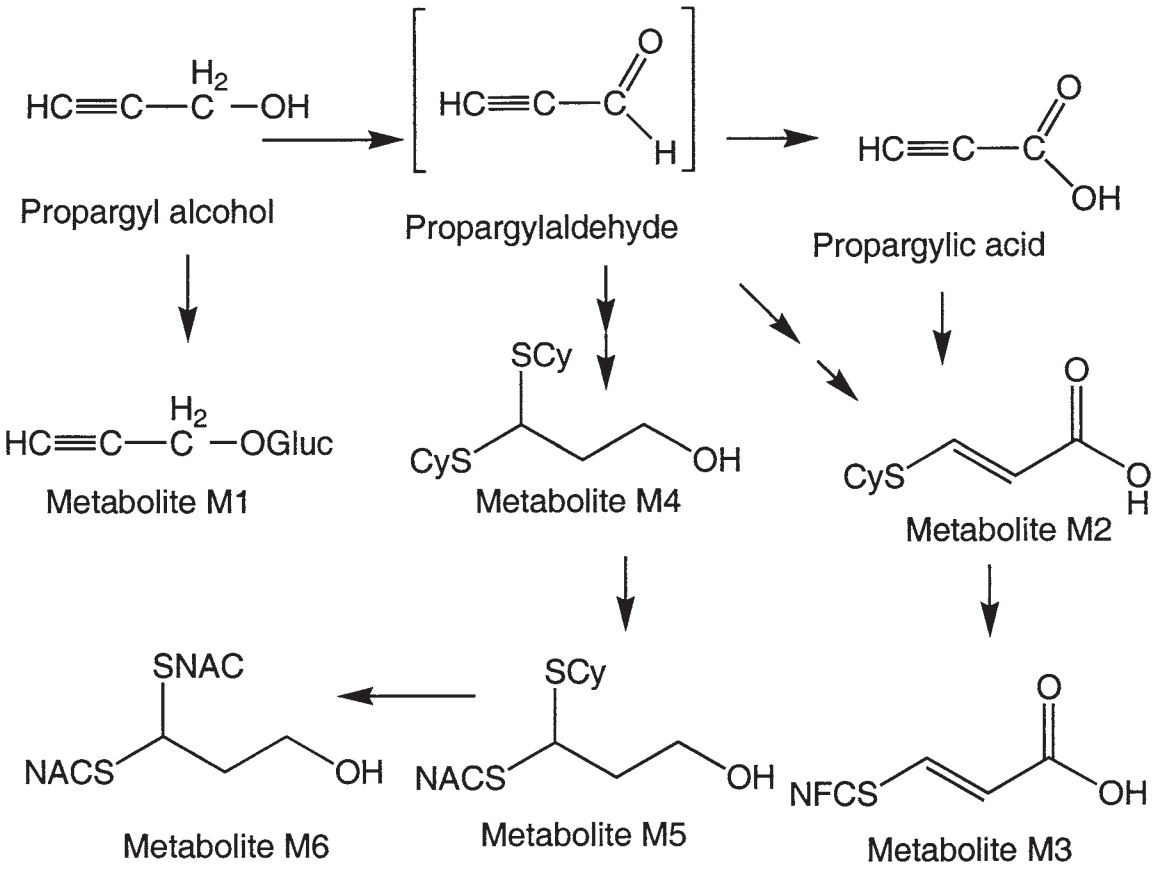


FIGURE 2
Metabolism of Propargyl Alcohol by Mice
SNAC, NACS=*N*-acetylcysteine; NFCS=*N*-formylcysteine; CyS=cysteine; Glu=glucuronic acid

1982). Acute dermal LD_{50}s reported for rabbits are 16 mg/kg (Rowe and McCollister, 1982) and 88 mg/kg (Vernot *et al*., 1977). A 1-hour acute inhalation LC_{50} is 1,040 ppm for female rats and 1,200 ppm for male rats (Vernot *et al*., 1977). A 2-hour inhalation exposure to 874 ppm was lethal to mice (Rowe and McCollister, 1982). Two out of three rats died after a 6-minute exposure to a saturated vapor of propargyl alcohol, and exposures of 12 minutes or longer were fatal to all exposed animals (Rowe and McCollister, 1982).

Propargyl alcohol is toxic to the liver and kidney of rats following repeated oral or inhalation exposure. In an inhalation study, male and female rats (strain not specified) were exposed to 80 ppm propargyl alcohol (7 hours/day, 5 days/week, for 59 exposure days over a 13-week period). At study termination, leukocyte counts and alanine aminotransferase activity were elevated in both sexes. Relative liver weights in males and females and relative kidney weights in females were elevated compared to controls. Microscopic changes included degenerative damage in the kidney and liver, which were more marked in females. In addition, there were fatty changes in the liver (Dow Chemical, 1964; Lington and Bevan, 1994).

Other subchronic inhalation studies, summarized in the BUA (1998) report on propargyl alcohol, have reported liver and kidney toxicity. In a preliminary study to a 90-day inhalation study, male and female Wistar rats were exposed to 0, 10, 50, or 200 ppm, 6 hours/day for 2 weeks (10 exposures). One female in the high dose group died. Body weight gain was significantly reduced in the 50 ppm males and in 200 ppm males and females. At study termination, the 200 ppm rats showed elevated alanine aminotransferase and alkaline phosphatase activities. The 200 ppm males had significantly increased relative liver weights, while the males and females in the 50 and 200 ppm groups had significantly increased relative kidney weights. Histopathological examination revealed lesions in the nasal mucosa (metaplasia was seen at 50 ppm) and the liver (hepatocellular hypertrophy, cytoplasmic granulation, and parenchymal single-cell necrosis at 200 ppm). In the subsequent 90-day inhalation study, male and female Wistar rats were exposed to 0, 1, 5, or 25 ppm, 6 hours/day for 90 days (65 exposures). The highest exposure concentration led to increased relative liver and kidney weights in both sexes. However, histopathological examination revealed no treatment-related findings.

Similar findings were observed in an oral gavage study in which male and female Sprague-Dawley rats were dosed with 0, 5, 15, or 50 mg/kg propargyl alcohol for 13 weeks. In the 15 and 50 mg/kg groups, hematological changes, changes in liver enzymes, increased liver and kidney weights, and histological changes in kidney (karyomegaly of renal tubular epithelial cells) and liver (megalocytosis, cytoplasmic vacuolation) were observed. No effects were observed at 5 mg/kg (Rubenstein *et al*., 1989; Lington and Bevan, 1994).

Propargyl alcohol had no systemic effects in male or female albino rabbits following repeated dermal application of 1, 3, 10, or 20 mg/kg to the intact or scarified skin for up to 91 days (GAF, 1965; Lington and Bevan, 1994). Dermal absorption is likely low due to the volatility of this material.

Humans

No information on the toxicity of propargyl alcohol in humans was found in the literature.

Reproductive and Developmental Toxicity

No information on the reproductive or developmental toxicity of propargyl alcohol in humans or experimental animals was found in the literature.

Carcinogenicity

No carcinogenicity studies in experimental animals or epidemiology studies in humans were found in the literature.

Genetic Toxicity

The mutagenic potential of propargyl alcohol has not been extensively investigated. Propargyl alcohol was reported to be nonmutagenic in standard tester strains of *Salmonella typhimurium*, with and without S9 activation enzymes (Basu and Marnett, 1984; Blakey *et al*., 1994). However, weak mutagenic activity was detected in the absence of S9 in strain 3052, a strain that is excision repair competent but lacks the pKM101 plasmid that promotes error-prone SOS DNA repair (Basu and Marnett, 1984). Significant dose-related increases in chromosomal aberrations were detected in cultured Chinese hamster ovary

cells exposed to propargyl alcohol in both the presence and the absence of S9 enzymes; the response in the presence of S9 was stronger than in the absence of activation (Blakey *et al*., 1994).

In vivo, no increases in the frequencies of micronucleated polychromatic erythrocytes (reticulocytes) were observed in bone marrow of male or female mice administered 24, 48, or 72 mg/kg propargyl alcohol by gavage in olive oil (Blakey *et al*., 1994). In this study, however, the highest dose, 72 mg/kg, was lethal, and therefore, data were obtained only from the two lower dose groups, neither of which showed evidence of having received a maximum tolerated dose (there was no evidence of bone marrow or overt toxicity in these two groups). Additional protocol deficiencies preclude considering these data a definitive assessment of the ability of propargyl alcohol to induce chromosomal alterations *in vivo*.

Study Rationale

Propargyl alcohol was nominated for carcinogenicity studies by the National Cancer Institute based on its high production volume, potential for occupational exposure, and lack of chronic toxicity data. The 2-week, 3-month, and 2-year studies were conducted in male and female F344/N rats and B6C3F1 mice to evaluate the toxicity and carcinogenicity of propargyl alcohol associated with whole-body inhalation exposure.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF PROPARGYL ALCOHOL

Propargyl alcohol was obtained from International Specialty Products (Texas City, TX) in one lot (04923BI) that was used in the 2-week, 3-month, and 2-year studies. Identity analyses were performed by the analytical chemistry laboratory, Midwest Research Institute (MRI, Kansas City, MO), and the study laboratory, Battelle Toxicology Northwest (Richland, WA). Purity analyses were performed by MRI and the study laboratory; elemental analysis was performed by Galbraith Laboratories, Inc. (Knoxville, TN) (Appendix I). Reports on analyses performed in support of the propargyl alcohol studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a colorless liquid, was identified as propargyl alcohol by infrared and proton nuclear magnetic resonance spectroscopy. Prior to the 2-week studies, elemental analyses for carbon, hydrogen, and oxygen were in agreement with the theoretical values for propargyl alcohol. Karl Fischer titration indicated 2,011 ppm water. High-performance liquid chromatography analysis indicated 0.17% formaldehyde. Purity by gas chromatography was determined to be greater than 99.0% by one system and 99.9% by a second system; a third system indicated a relative purity exceeding 99.6% compared to a reference standard. The overall purity of lot 04923BI was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored at 2° to 8° C in the original shipping containers (55-gallon metal drums). Stability was monitored using gas chromatography. No degradation of the bulk chemical was detected.

VAPOR GENERATION AND EXPOSURE SYSTEM

Propargyl alcohol was pumped onto glass beads in a heated glass column where it was vaporized. Heated nitrogen flowed through the column and carried the vapor to a short vapor distribution manifold, where concentration was controlled by the chemical pump and nitrogen flow rates. Pressure in the distribution manifold was fixed to ensure constant flows through the manifold and into the chambers.

Individual Teflon® delivery lines carried the vapor from the manifold to three-way exposure valves at the chamber inlets. The exposure valves diverted vapor delivery to the exposure chamber exhaust until the generation system stabilized. The flow rate to each chamber was controlled by a metering valve at the manifold. The chamber exposure valves were rotated to allow the vapor to flow to each exposure chamber inlet duct where it was diluted with conditioned chamber air to achieve the desired exposure concentration.

The study laboratory designed the inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A small-particle detector was used with and without animals in the exposure chambers to ensure that propargyl alcohol vapor, and not aerosol, was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm^3) were detected.

VAPOR CONCENTRATION MONITORING

Chamber and room concentrations of propargyl alcohol were monitored by an on-line gas chromatograph. Samples were drawn from each exposure chamber approximately every 20 (2-week and 3-month studies) or 24 (2-year studies) minutes during each 6-hour exposure period using Hasteloy-C stream-select and gas-sampling valves in a separate, heated valve oven.

The on-line gas chromatograph was checked throughout the day for instrument drift against an on-line standard of propargyl alcohol in nitrogen supplied by a standard generator. The on-line gas chromatograph was calibrated prior to the start of each study, three times during

the 2-week studies, and monthly during the 3-month and 2-year studies by a comparison of chamber concentration data to data from grab samples that were collected with adsorbent gas sampling tubes containing silica gel, extracted with methanol containing 4-methyl-2-pentanol as an internal standard, and analyzed using an off-line gas chromatograph. The volumes of gas were sampled at a constant flow rate ensured by a calibrated critical orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standards of propargyl alcohol and the internal standard (4-methyl-2-pentanol) in methanol.

Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 12.5 minutes. Based on experimental data, a T_{90} value of 12 minutes was selected for the 2-week and 3-month studies and 14 minutes for the 2-year studies.

Evaluations of chamber uniformity and persistence and monitoring for propargyl alcohol impurities were conducted periodically throughout the studies by gas chromatography. Chamber uniformity was maintained; no degradation was detected.

2-Week Studies

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, rats and mice were 4 to 5 weeks old. Rats and mice were quarantined for 11 days and were approximately 5 to 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice were exposed to propargyl alcohol at concentrations of 0, 31.3, 62.5, 125, 250, or 500 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 16 (rats) or 17 (mice) days. Exposure concentrations were selected after review of a 14-day study conducted by industry using 200 ppm as the highest exposure concentration (BUA, 1998). Feed was available *ad libitum* except during exposure periods; water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded daily. The animals were weighed initially, on days 6 and 13, and at the end of the studies. Blood was collected from five male and five female chamber control rats and mice at the end of the studies, and the sera were analyzed according to the protocols of the Sentinel Animal Program (Appendix K). Details of the study design and animal maintenance are summarized in Table 2.

Necropsies were performed on all rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations were performed on all chamber control and 500 ppm rats and mice. Table 2 lists the tissues and organs examined.

3-Month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to propargyl alcohol and to determine the appropriate exposure concentrations to be used in the 2-year studies.

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 11 or 12 (female rats) days and were 5 to 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At week 1 and at the end of the studies, serologic analyses were performed on five male and five female sentinel or chamber control rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were exposed to propargyl alcohol at concentrations of 0, 4, 8, 16, 32, or 64 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. Additional groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days for clinical pathology analyses. Feed was available *ad libitum* except during exposure and urine collection periods; water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded weekly for rats and mice. The animals were weighed initially, weekly,

and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Animals were anesthetized with carbon dioxide, and blood was collected from the retroorbital sinus of clinical pathology rats on days 3 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats only) analyses. Blood samples for hematology analyses were placed in tubes containing potassium EDTA. Packed cell volume; hemoglobin concentration; erythrocyte, platelet, and leukocyte counts; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined using an ABX Cobas Helios (ABX Co., Irvine, CA). Manual hematocrit values were determined using a microcentrifuge (Heraeus Haemofuge; Germany) and a Damon/IEC capillary reader (International Equipment Co., Needham Heights, MA) for comparison to Helios values for packed cell volume. Blood smears were stained with Romanowsky-type aqueous stain in a Wescor 1700 aerospray slide stainer (Wescor, Inc., Logan, UT). Leukocyte differential counts were based on classifying a minimum of 100 white cells. Reticulocytes were stained with new methylene blue and enumerated as a reticulocyte:erythrocyte ratio using the Miller disc method (Brecher and Schneiderman, 1950). Blood samples for clinical chemistry analyses were placed in tubes without anticoagulant and containing a separator gel, allowed to clot, and centrifuged. Parameters were determined using Roche Cobas Fara methodologies. After three consecutive exposure days during week 12, male and female core study rats were placed in metabolism cages, and urine was collected over ice for 16 hours. After the volume and specific gravity were measured, the urine samples were centrifuged and aliquots collected for determination of aspartate aminotransferase, alkaline phosphatase, creatinine, glucose, protein, lactate dehydrogenase, and *N*-acetyl-β-D-glucosaminidase using Roche Cobas Fara methodologies. Creatinine and urine γ-glutamyltransferase were determined using a Roche Hitachi 912 system (Roche Diagnostics Corp., Indianapolis, IN) with reagents supplied by the manufacturer. Table 2 lists the parameters measured.

At the end of the 3-month studies, samples were collected for sperm count and motility and vaginal cytology evaluations on core study rats and mice exposed to 0, 16, 32, or 64 ppm. The parameters evaluated are listed in Table 2. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on the 0 and 64 ppm core study rats and mice. Table 2 lists the tissues and organs routinely examined.

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were exposed to propargyl alcohol by inhalation at concentrations of 0, 8 (mice only), 16, 32, or 64 (rats only) ppm, 6 hours plus T_{90} (14 minutes) per day, 5 days per week for 105 weeks.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc., for use in the 2-year studies. Animals were quarantined for 11 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were 5 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats and mice were housed individually. Feed was available *ad libitum* except during exposure periods. Water was available *ad libitum*. Cages and racks were changed weekly. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Body weights were recorded initially, weekly for the first 13 weeks, and every 4 weeks through week 93, then every 2 weeks, and at the end of the studies. Clinical findings were recorded every 4 weeks through week 93, then every 2 weeks, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all rats and mice. 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 2.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. 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. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the nose of male and female rats and mice and the eye of male and female mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of Propargyl Alcohol

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory		
Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)
Strain and Species		
F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source		
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
11 days	Rats: 11 (males) or 12 (females) days Mice: 11 days	11 days
Average Age When Studies Began		
5 to 6 weeks	5 to 6 weeks	5 to 7 weeks
Date of First Exposure		
September 11, 2000	Rats: November 13 (males) or 14 (females), 2000 Mice: November 13, 2000	Rats: October 1, 2001 Mice: September 17, 2001
Duration of Exposure		
6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 16 (rats) or 17 (mice) days	6 hours per day plus T_{90} (12 minutes), 5 days per week, for 14 weeks	6 hours plus T_{90} (14 minutes) per day, 5 days per week, for 105 weeks
Date of Last Exposure		
Rats: September 26, 2000 Mice: September 27, 2000	Rats: February 12 (males) or 13 (females), 2001 Mice: February 14 (males) or 15 (females), 2001	Rats: September 28-October 1, 2003 Mice: September 14-September 18, 2003
Necropsy Dates		
Rats: September 27, 2000 Mice: September 28, 2000	Rats: February 13 (males) or 14 (females), 2001 Mice: February 15 (males) or 16 (females), 2001	Rats: September 29-October 2, 2003 Mice: September 15-19, 2003
Average Age at Necropsy		
8 to 9 weeks	19 to 20 weeks	109 to 111 weeks
Size of Study Groups		
5 males and 5 females	10 males and 10 females	50 males and 50 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage		
1	1	1

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of Propargyl Alcohol

2-Week Studies	3-Month Studies	2-Year Studies
Method of Animal Identification		
Tail tattoo	Tail tattoo	Tail tattoo
Diet		
Irradiated NTP-2000 open formula wafer diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, except during exposure periods, changed weekly	Same as 2-week studies. Available *ad libitum* except during exposure and urine collection periods.	Same as 2-week studies
Water		
Tap water (City of Richland municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 2-week studies	Same as 2-week studies
Cages		
Stainless steel wire-bottom (Lab Products, Inc., Seaford, DE), changed weekly	Same as 2-week studies	Same as 2-week studies
Chamber Air Supply Filters		
Single HEPA (Environmental Filter, Santa Rosa, CA); charcoal (RSE, Inc., New Baltimore, MI); Purafil (Environmental Systems, Lynnwood, WA)	Same as 2-week studies	Same as 2-week studies, except HEPA filters open stock and changed annually
Chambers		
Stainless steel with excreta pan suspended below each cage unit (Lab Products, Inc., Seaford, DE), changed weekly	Same as 2-week studies	Same as 2-week studies
Chamber Environment		
Temperature: 75° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour	Temperature: 75° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour	Temperature: 75° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour
Exposure Concentrations		
0, 31.3, 62.5, 125, 250, or 500 ppm	0, 4, 8, 16, 32, or 64 ppm	Rats: 0, 16, 32, or 64 ppm Mice: 0, 8, 16, or 32 ppm
Type and Frequency of Observation		
Observed twice daily; animals were weighed initially, on days 6 and 13, and at the end of the studies; clinical findings were recorded daily.	Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies. Clinical findings were recorded weekly.	Observed twice daily; animals were weighed initially, weekly for 13 weeks, every 4 weeks through week 93, and then every 2 weeks and at the end of the studies. Clinical findings were recorded every 4 weeks through week 93, then every 2 weeks, and at the end of the studies.
Method of Sacrifice		
CO_2 asphyxiation	Same as 2-week studies	Same as 2-week studies

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of Propargyl Alcohol

2-Week Studies	3-Month Studies	2-Year Studies
Necropsy		
Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testes, and thymus.	Necropsies were performed on all core study animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology		
None	Blood was collected from the retroorbital sinus of clinical pathology rats on days 3 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats only). Core study rats were placed in metabolism cages for 16-hour urine collection during week 12. ***Hematology:*** hematocrit; packed red cell volume; hemoglobin; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, globulin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, cholinesterase, and bile acids ***Urinalysis:*** creatinine, glucose, protein, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, γ-glutamyltransferase, *N*-acetyl-β-D-glucosaminidase, volume, and specific gravity	None

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of Propargyl Alcohol

2-Week Studies	3-Month Studies	2-Year Studies
Histopathology		
Histopathology was performed on 0 and 500 ppm rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined to the no-effect level: left kidney, liver, lung, and nose.	Complete histopathology was performed on 0 and 64 ppm core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined to the no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland (except male mice), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathology was performed on all animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Motility and Vaginal Cytology		
None	At the end of the studies, sperm samples were collected from core study male animals in the 0, 16, 32, and 64 ppm groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis, per gram testis, per cauda, and per gram cauda and epididymal spermatozoal motility. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from core study females exposed to 0, 16, 32, or 64 ppm for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. 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 of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B4, C1, C4, D1, and D3 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., Harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, urinalysis, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley

(1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations. Proportions of regularly cycling females in each exposure group were compared to the control group using Fisher's exact test (Gart *et al.*, 1979). Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each exposure group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within estrus and diestrus. Equality of transition matrices among exposure groups and between the chamber control group and each exposed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The current NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year 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 Technical 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 Technical Report.

Genetic Toxicology

The genetic toxicity of propargyl alcohol was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al*., 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller,

1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.,* 1987; Zeiger *et al.,* 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.,* 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

RATS

2-WEEK STUDY

All males exposed to 250 or 500 ppm and all females exposed to 500 ppm died after one day of exposure; all males exposed to 125 ppm and all females exposed to 250 ppm died by the end of the third day of exposure; and one 125 ppm female died on day 5 (Table 3). Final mean body weights and body weight gains were significantly decreased in 62.5 ppm males and 125 ppm females. Lethargy, ataxia, abnormal breathing, and nasal/eye discharge were observed in the 125 and 250 ppm groups. One 62.5 ppm female had nasal/eye discharge on day 2.

Absolute and relative right kidney weights of 62.5 and 125 ppm females were significantly greater than those of the chamber controls (Table G1). Absolute and relative liver weights of 125 ppm females were significantly increased. Dark livers were noted in most 250 and 500 ppm males and females at necropsy, and crusted noses were noted in four 125 ppm males.

Livers of males and females exposed to 250 or 500 ppm contained large areas of degeneration and necrosis of hepatocytes, accompanied by marked congestion of affected areas (Table 4). The predominant pattern was periportal, with some larger degenerated and necrotic areas covering the major portion of the affected lobules.

TABLE 3
Survival and Body Weights of Rats in the 2-Week Inhalation Study of Propargyl Alcohol

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	89 ± 2	162 ± 4	73 ± 3	
31.3	5/5	88 ± 3	151 ± 5	64 ± 4	94
62.5	5/5	89 ± 3	144 ± 4*	55 ± 3**	89
125	0/5[c]	89 ± 3	—	—	—
250	0/5[d]	89 ± 2	—	—	—
500	0/5[d]	89 ± 3	—	—	—
Female					
0	5/5	80 ± 2	121 ± 1	41 ± 2	
31.3	5/5	80 ± 2	115 ± 2	35 ± 1	96
62.5	5/5	80 ± 2	116 ± 3	36 ± 2	96
125	4/5[e]	81 ± 2	111 ± 4*	31 ± 4*	92
250	0/5[f]	80 ± 1	—	—	—
500	0/5[d]	81 ± 2	—	—	—

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Days of death: 2, 2, 2, 3, 3
[d] Day of deaths: 1
[e] Day of death: 5
[f] Days of death: 1, 1, 1, 2, 2

TABLE 4
Incidences of Nonneoplastic Lesions of the Liver in Rats in the 2-Week Inhalation Study of Propargyl Alcohol

	Chamber Control	31.3 ppm	62.5 ppm	125 ppm	250 ppm	500 ppm
Male						
Number Examined Microscopically	5	0	5	5	5	5
Hepatocytes, Necrosis, Periportal[a]	0		0	0	5** (3.0)[b]	5** (3.0)
Congestion, Periportal	0		0	0	5** (4.0)	5** (4.0)
Erythrophagocytosis, Periportal	0		0	0	5** (3.0)	5** (3.0)
Hepatocytes, Necrosis, Centrilobular	0		0	5** (4.0)	0	0
Congestion, Centrilobular	0		0	5** (3.0)	0	0
Female						
Number Examined Microscopically	5	0	5	5	5	5
Hepatocytes, Necrosis, Periportal	0		0	0	5** (3.0)	5** (3.0)
Congestion, Periportal	0		0	0	5** (4.0)	5** (4.0)
Erythrophagocytosis, Periportal	0		0	0	5** (3.0)	5** (3.0)
Hepatocytes, Necrosis, Centrilobular	0		0	1 (4.0)	0	0
Congestion, Centrilobular	0		0	1 (3.0)	0	0
Hepatocytes, Cytoplasmic Vacuolization, Centrilobular	0		0	4* (1.5)	0	0

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

Some affected hepatocytes had karyolytic or pyknotic nuclei, and many had large pale vacuoles distending the cytoplasm. Necrosis was graded as moderate because some normal hepatocytes remained in affected areas; congestion was graded as marked. Many Kupffer cells and hepatocytes appeared to be distended by the presence of erythrocytes in the cytoplasm; this lesion was classified as erythrophagocytosis.

Males and females exposed to 125 ppm also had microscopic hepatic lesions, but the morphologic pattern and severity differed from those dying earlier in the study. All males exposed to 125 ppm exhibited marked necrosis of hepatocytes and moderate congestion predominantly in the centrilobular areas; in many areas, however, the necrosis extended into the midzonal region, sometimes with bridging necrosis between adjacent terminal venules and sometimes with involvement of entire lobules. The incidence of hepatic necrosis in females exposed to 125 ppm was lower than in males; the female from this group that died after 5 days of exposure had marked centrilobular hepatocyte necrosis similar to that noted in the 125 ppm males. The remaining four females had only multifocal vacuolization of centrilobular hepatocytes, varying in severity from minimal to moderate.

Exposure Concentration Selection Rationale: Based on the decreased survival of males and females exposed to 125 ppm or greater, the propargyl alcohol exposure concentrations selected for the 3-month inhalation study in rats were 4, 8, 16, 32, and 64 ppm. Although final mean body weights were decreased in males exposed to 62.5 ppm, decreased body weight gain occurred primarily during the first week of the study with recovery observed during the second week of the study.

3-MONTH STUDY

All rats survived to the end of the study (Table 5). Final mean body weights and body weight gains of all exposed groups were similar to those of the chamber control groups. There were no clinical findings related to propargyl alcohol exposure.

Hematology, clinical chemistry, and urinalysis data for rats in the 3-month inhalation study of propargyl alcohol are shown in Tables 6 and/or F1. The most dramatic change involved serum cholinesterase activity. An exposure concentration- and time-related decrease in serum cholinesterase activity occurred in exposed male and female rats; females were more affected. For example, females in the 64 ppm group demonstrated a 19%, 42%, and 50% decrease in cholinesterase activity on days 3 and 23 and at week 14, respectively. Males in the 64 ppm group demonstrated only 15% and 27% decreases on day 23 and at week 14, respectively. In an attempt to identify a direct-acting enzyme inhibitory effect of the parent compound, propargyl alcohol was added to normal rat serum (final concentrations of 0, 0.1, 1, and 10 mM), incubated at room temperature for various time periods (up to 2.5 hours), and analyzed for cholinesterase activity by two methods utilizing either propionylthiocholine or butyrylthiocholine as substrates. For the concentrations tested, addition of propargyl alcohol to rat serum did not result in any significant inhibition of cholinesterase activity (data not shown). Since rat serum contains acetylcholinesterase and butyrylcholinesterase activity, an attempt to differentiate whether one or both enzyme fractions were affected was undertaken. Dr. Stephanie Padilla (U.S. Environmental Protection Agency) analyzed the male and female serum samples collected at study termination. To ensure sample stability, the samples were first analyzed using the same substrate (acetylthiocholine) as was used for the original analysis. Original and repeat analysis results were compared and indicated the samples were stable. To determine if one or both enzymes were affected by exposure of animals to propargyl alcohol, the samples were then

TABLE 5
Survival and Body Weights of Rats in the 3-Month Inhalation Study of Propargyl Alcohol

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	95 ± 2	323 ± 4	228 ± 4	
4	10/10	95 ± 3	339 ± 7	245 ± 8	105
8	10/10	96 ± 3	317 ± 8	221 ± 8	98
16	10/10	94 ± 2	322 ± 9	228 ± 9	100
32	10/10	93 ± 2	319 ± 3	226 ± 2	99
64	10/10	93 ± 3	314 ± 7	222 ± 7	97
Female					
0	10/10	86 ± 3	192 ± 4	105 ± 3	
4	10/10	88 ± 2	197 ± 6	109 ± 4	103
8	10/10	84 ± 1	184 ± 4	100 ± 3	96
16	10/10	86 ± 2	186 ± 4	100 ± 3	97
32	10/10	89 ± 2	191 ± 3	102 ± 3	98
64	10/10	88 ± 2	184 ± 3	96 ± 4	96

[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test.

TABLE 6
Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm	64 ppm
n	10	10	10	10	10	10
Male						
Cholinesterase (IU/L)						
Day 3	1,094.0 ± 24.0	1,045.0 ± 16.0	1,065.0 ± 13.0	1,046.0 ± 18.0	1,076.0 ± 19.0	1,142.0 ± 29.0
Day 23	991.2 ± 34.4	964.8 ± 46.1	948.5 ± 34.3	934.2 ± 25.0	878.5 ± 29.7**	839.4 ± 27.0**
Week 14	1,071.0 ± 26.2	1,085.5 ± 67.4	1,045.8 ± 30.6	1,100.5 ± 41.2	897.7 ± 25.5**	777.8 ± 25.3**
Female						
Cholinesterase (IU/L)						
Day 3	1,999.0 ± 171.0	1,728.0 ± 85.0	1,890.0 ± 107.0	1,801.0 ± 90.0	1,504.0 ± 56.0*	1,614.0 ± 52.0
Day 23	4,407.4 ± 146.7	3,863.2 ± 194.8	4,298.4 ± 177.0	3,843.2 ± 115.9*	3,125.7 ± 155.1**[b]	2,558.3 ± 86.6**
Week 14	7,845.9 ± 153.0	7,612.5 ± 181.6	7,001.5 ± 309.6*	6,921.3 ± 185.4**	6,341.3 ± 189.1**	3,900.1 ± 186.5**

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

analyzed using specific inhibitors for butyrylcholinesterase (iso-OMPA); results suggested that butyrylcholinesterase was inhibited (data not shown). Changes in other hematology or chemistry variables were minor and not considered toxicologically relevant.

Absolute liver weight was significantly increased in 64 ppm males (Table G2). Relative liver and kidney weights were significantly increased in 64 ppm males and females and in 32 ppm males, and relative liver weight was significantly increased in 16 ppm males. There were no significant differences between exposed groups and chamber controls in reproductive tissue evaluations in males or vaginal cytology parameters in females (Tables H1 and H2).

Exposure-related histopathology findings were limited to the nose (Table 7). Minimal to mild hyperplasia of respiratory epithelium of the nose was noted in all exposed groups and in two of the male controls; the incidences were significantly increased in all exposed groups except 8 ppm males and 4 ppm females. Respiratory hyperplasia was characterized by increased numbers of columnar epithelial cells lining the dorsal meatus of Levels I and/or II and the presence of occasional dilated glands in the underlying lamina propria. Squamous metaplasia of the respiratory epithelium was noted in a few males and most females exposed to 64 ppm. Necrosis of respiratory epithelium was also noted in two 64 ppm females. These changes were noted in the septum, turbinates, and lateral walls of Level I. Necrosis of olfactory epithelium was present in half of the males and females exposed to 64 ppm and in a few males and females exposed to 32 ppm. The necrosis was present on the dorsal septum and adjacent tips of the turbinates of Level III. There was sloughed olfactory epithelium with pyknotic nuclei, and only a thin layer of flattened or vacuolated basal cells remained on the surfaces of affected areas. The transition from normal to necrotic olfactory epithelium was usually abrupt.

Exposure Concentration Selection Rationale: Because there were no significant changes in survival or body weight of males or females, the propargyl alcohol exposure concentrations selected for the 2-year inhalation study in rats were 16, 32, and 64 ppm. Incidences of nasal lesions in the respiratory and olfactory epithelium were increased at 32 ppm and greater, but the lesions were minimal to mild in severity and were not considered to be life threatening.

TABLE 7
Incidences of Nonneoplastic Lesions of the Nose in Rats in the 3-Month Inhalation Study of Propargyl Alcohol

	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm	64 ppm
Male						
Number Examined Microscopically	10	10	10	10	10	10
Respiratory Epithelium, Hyperplasia[a]	2 (1.0)[b]	7* (1.1)	5 (1.2)	7* (1.0)	9** (1.4)	10** (1.6)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	0	3 (1.3)
Olfactory Epithelium, Necrosis	0	0	0	0	2 (1.0)	5* (1.8)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Respiratory Epithelium, Hyperplasia	0	2 (1.0)	4* (1.0)	4* (1.0)	10** (1.0)	9** (1.1)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	0	8** (1.1)
Respiratory Epithelium, Necrosis	0	0	0	0	0	2 (1.0)
Olfactory Epithelium, Necrosis	0	0	0	0	3 (2.0)	5* (1.4)

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

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 8 and in the Kaplan-Meier survival curves (Figure 3). Survival of 32 and 64 ppm males was significantly less than that of the chamber control group.

Body Weights and Clinical Findings

Mean body weights of males exposed to 64 ppm were less than those of the chamber controls after week 24 of the study (Figure 4; Tables 9 and 10). Mean body weights of exposed females were similar to those of the chamber controls.

Nine 64 ppm males had torso/ventral masses, and six 64 ppm males had torso/ventral ulcers/abscesses, but these lesions were seen in only two chamber control males. An increased incidence of eye abnormality (9/50) was observed in the 32 ppm female group compared to controls (3/50).

Clonic seizures of short duration, usually observed during routine animal care, were noted in a few chamber control and exposed males (0 ppm, 1/50; 16 ppm, 5/50; 32 ppm, 4/50; 64 ppm, 2/50) and females (3/50, 3/50, 6/50, 9/50). More females (21) than males (12) developed seizures, and there were increased incidences of seizures with increasing exposure concentration in females. The seizures were initially observed during week 42. No evidence of brain lesions was found to account for the cause or effect of the seizures. Similarly, sporadic seizures have been observed in F344/N rats in six other NTP inhalation or dermal studies at three different laboratories. In all of these studies, the single common factor was that the animals were housed individually. No such episodes have been observed in concurrent dosed feed, gavage, or drinking water studies in which rats are group housed. In the individually housed animals, most seizures were observed early in the day, when technical and maintenance activities were commencing following the animals' dark cycle period. No deaths were associated with the seizures, and there were no correlations with body weight, feed consumption or composition, or histopathological lesions in this or the other studies. Thus, these transient events were not considered to have affected the toxicologic or carcinogenic evaluations of this study.

TABLE 8
Survival of Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Male				
Animals initially in study	50	50	50	50
Missexed[a]	1	0	0	0
Moribund	20	19	26	30
Natural deaths	2	8	9	4
Animals surviving to study termination	27[b]	23	15	16
Percent probability of survival at end of study[c]	55	46	30	32
Mean survival (days)[d]	683	672	661	654
Survival analysis[e]	P=0.014	P=0.467	P=0.025	P=0.026
Female				
Animals initially in study	50	50	50	50
Moribund	18	16	19	19
Natural deaths	3	3	4	5
Animals surviving to study termination	29	31[f]	27	26
Percent probability of survival at end of study	58	62	54	52
Mean survival (days)	678	697	686	685
Survival analysis	P=0.515	P=0.663N	P=1.000	P=0.818

[a] Censored from survival analysis
[b] Includes one moribund animal that was removed during the last week of the study.
[c] Kaplan-Meier determinations
[d] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[e] The result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns. A lower mortality in an exposed group is indicated by **N**.
[f] Includes one animal that died during the last week of the study.

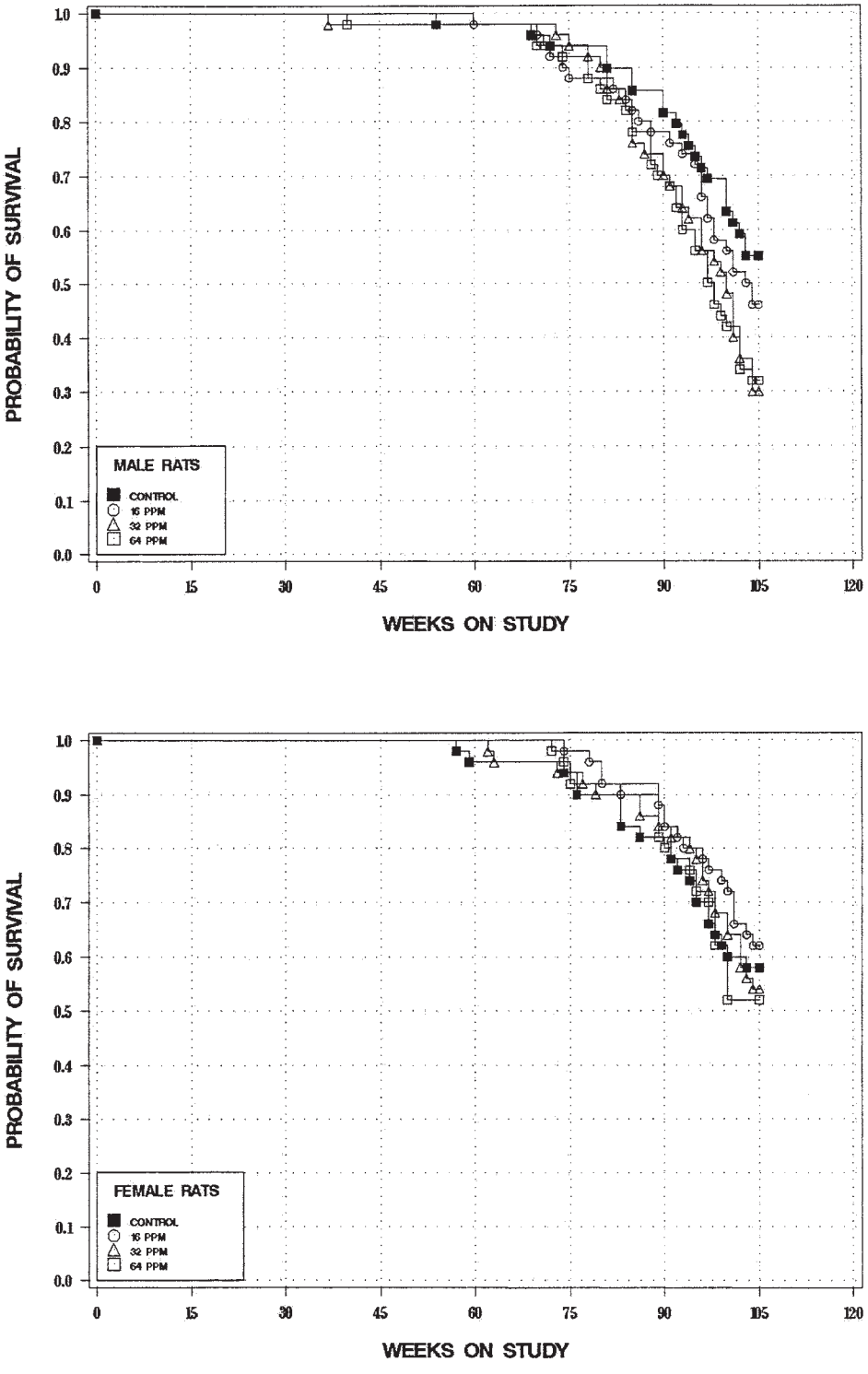


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Propargyl Alcohol by Inhalation for 2 Years

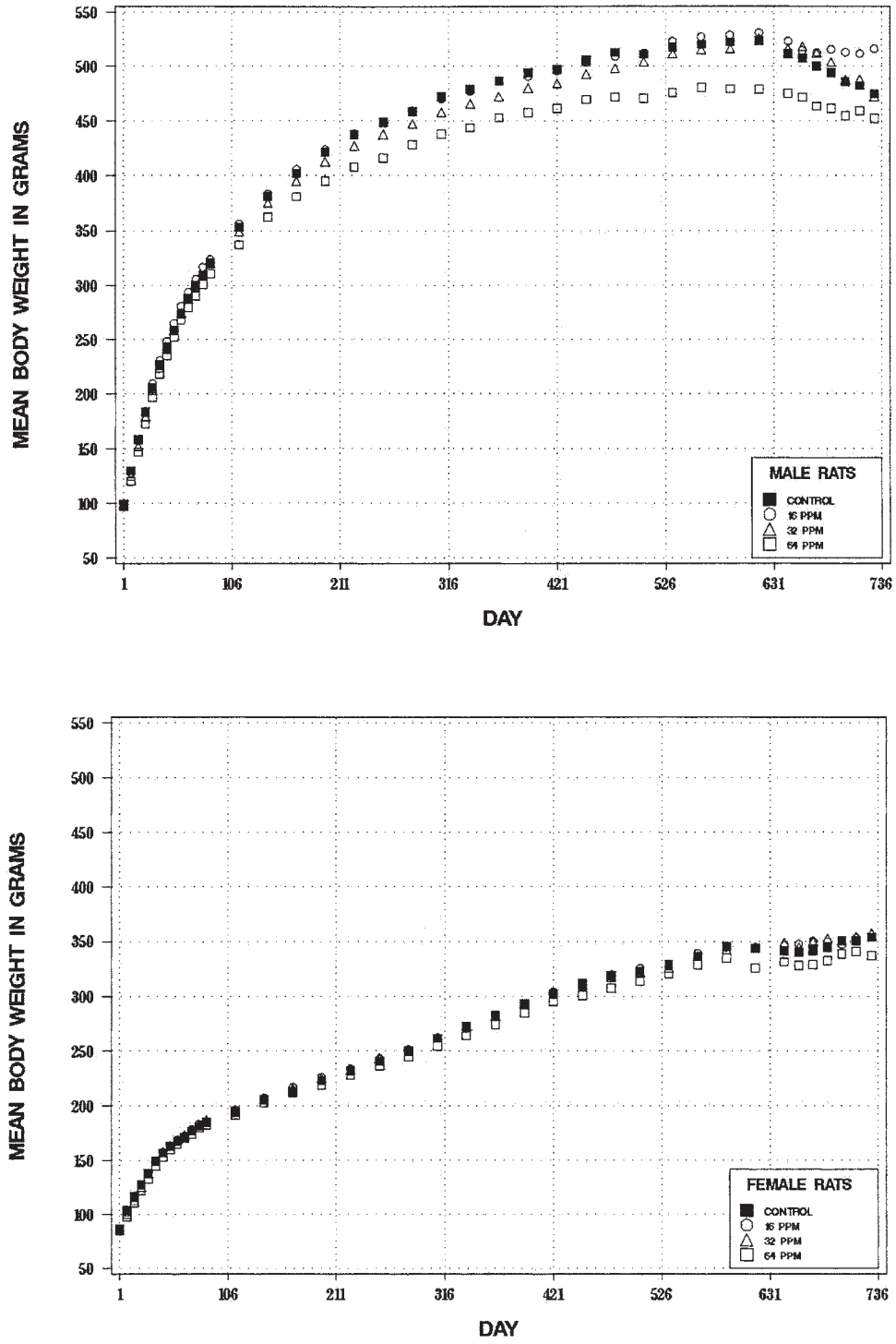


FIGURE 4
Growth Curves for Male and Female Rats
Exposed to Propargyl Alcohol by Inhalation for 2 Years

TABLE 9
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol

Days on Study	Chamber Control		16 ppm			32 ppm			64 ppm		
	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	99	49	99	100	50	98	99	50	98	98	50
8	130	49	128	98	50	125	96	50	120	93	50
15	158	49	157	99	50	152	96	50	147	93	50
22	183	49	184	101	50	179	98	50	172	94	50
29	206	49	209	102	50	203	99	50	197	96	50
36	226	49	230	102	50	224	99	50	218	97	50
43	243	49	248	102	50	241	99	50	235	97	50
50	259	49	265	102	50	258	100	50	252	98	50
57	273	49	280	103	50	275	100	50	268	98	50
64	288	49	293	102	50	287	100	50	279	97	50
71	300	49	305	102	50	299	100	50	290	97	50
78	309	49	316	103	50	308	100	50	300	97	50
85	321	49	323	101	50	319	100	50	310	97	50
113	353	49	356	101	50	349	99	50	337	96	50
141	381	49	384	101	50	375	99	50	362	95	50
169	402	49	406	101	50	395	98	50	381	95	50
197	421	49	424	101	50	413	98	50	395	94	50
225	438	49	438	100	50	427	98	50	408	93	50
253	449	49	449	100	50	438	98	50	416	93	50
281	459	49	459	100	50	447	98	49	429	93	49
309	472	49	470	100	50	458	97	49	438	93	49
337	479	49	477	100	50	466	97	49	444	93	49
365	486	49	486	100	50	472	97	49	453	93	49
393	494	48	491	99	50	480	97	49	458	93	49
421	497	48	496	100	49	485	98	49	462	93	49
449	506	48	503	100	49	493	98	49	469	93	49
477	513	48	509	99	49	498	97	49	472	92	49
505	511	46	512	100	46	504	99	49	471	92	47
533	518	46	523	101	44	511	99	47	476	92	46
561	522	45	527	101	44	516	99	45	480	92	43
589	525	42	532	101	41	517	99	41	479	91	41
617	524	42	531	101	39	526	100	37	484	92	35
645	511	39	523	102	38	522	102	32	477	93	31
659	512	36	514	101	36	518	101	31	472	92	30
673	503	34	512	102	33	512	102	28	467	93	27
687	494	34	515	104	29	504	102	27	462	94	23
701	486	31	521	107	26	492	101	23	455	94	21
715	482	29	512	106	26	488	101	18	459	95	17
Mean for weeks											
1-13	230		234	101		228	99		222	96	
14-52	428		429	100		419	98		401	94	
53-103	505		513	102		502	100		469	93	

TABLE 10
Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of Propargyl Alcohol

Days on Study	Chamber Control		16 ppm			32 ppm			64 ppm		
	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	87	50	86	99	50	86	99	50	85	98	50
8	104	50	102	98	50	101	97	50	98	94	50
15	117	50	116	99	50	114	97	50	111	95	50
22	128	50	127	100	50	126	99	50	122	96	50
29	138	50	138	100	50	138	100	50	132	96	50
36	150	50	149	100	50	150	100	50	145	97	50
43	157	50	157	100	50	158	101	50	153	98	50
50	163	50	164	100	50	163	100	50	160	98	50
57	167	50	169	101	50	169	101	50	165	99	50
64	171	50	173	101	50	173	101	50	170	99	50
71	177	50	178	101	50	177	100	50	174	99	50
78	181	50	183	101	50	183	101	50	180	99	50
85	185	50	185	100	50	187	101	50	183	99	50
113	195	50	196	101	50	194	100	50	191	98	50
141	205	50	207	101	50	207	101	50	203	99	50
169	213	50	217	102	50	216	101	50	212	100	50
197	223	50	226	101	50	225	101	50	219	98	50
225	232	50	233	101	50	233	101	50	228	99	50
253	241	50	243	101	50	244	101	50	236	98	50
281	250	50	251	100	50	251	100	50	244	98	50
309	262	50	262	100	50	262	100	50	254	97	50
337	273	50	271	99	50	273	100	50	264	97	50
365	282	50	282	100	50	282	100	50	274	97	50
393	293	49	293	100	50	293	100	50	285	97	50
421	302	48	304	100	50	303	100	50	295	98	50
449	311	48	310	100	50	310	100	48	301	97	50
477	319	48	319	100	50	318	100	48	307	97	50
505	323	48	325	101	50	322	100	48	314	97	49
533	329	45	329	100	49	326	99	47	320	97	46
561	336	45	339	101	46	337	100	45	329	98	46
589	345	42	344	100	45	342	99	45	334	97	46
617	344	41	345	100	44	344	100	43	326	95	45
645	341	38	349	102	40	349	102	41	331	97	39
659	343	36	347	101	40	345	101	40	328	95	38
673	347	33	350	101	39	350	101	37	328	95	36
687	344	32	349	101	38	353	102	34	333	97	31
701	350	30	347	99	35	350	100	32	338	97	26
715	353	29	352	100	33	354	100	29	341	96	26
Mean for weeks											
1-13	148		148	100		148	100		145	97	
14-52	233		234	101		234	101		228	98	
53-103	329		330	100		330	100		318	97	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia and neoplasms and/or nonneoplastic lesions of the nose and preputial gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Nose: Respiratory epithelial adenomas were present in three 64 ppm males and one 32 ppm female (Tables 11, A2, and B1). Although neither incidence reached statistical significance, there was a significant dose-related trend in males, and the incidence in 64 ppm males exceeded the historical control ranges for inhalation studies and all study routes (Tables 11 and A3a). Respiratory adenomas are rare tumors in male rats, having occurred in only 1/447 (0.2%) chamber controls in inhalation studies and in 2/1,439 (0.1%) controls from all study routes. These adenomas were well-demarcated, polypoid masses arising from the nasoturbinate, maxilloturbinate, or lateral wall in Level I (Plate 1). Microscopically, they presented as sessile or pedunculated, polypoid proliferations of well-differentiated respiratory glands and hyperplastic surface epithelium without cytologic atypia.

One 16 ppm female was found to have a carcinoma (Tables 11 and B1) located in the region of Steno's glands and the maxillary sinus in Level III. Steno's glands surround the maxillary sinuses in Level III and are histologically and topographically distinct from the respiratory glands and the respiratory epithelium of Levels I and II where the respiratory adenomas arise. This tumor formed a mass composed of closely packed solid nests and small glands lined by epithelial cells with a high nuclear/cytoplasmic ratio and moderate mitotic activity. The tumor mass partially compressed the nasopharyngeal duct medially, invaded dorsally into the ventral portion of the ethmoid turbinates, and invaded laterally into a paraosseous vein and perineural space.

A spectrum of nonneoplastic changes was observed in the respiratory and olfactory epithelium at all levels of exposure (Tables 11, A4, and B4). All exposed groups had significantly increased incidences of respiratory epithelial hyperplasia and respiratory glandular hyperplasia in Levels I and II. Microscopically, the respiratory epithelium lining the lateral meatus and tips of the turbinates was thickened due to an increased number of cell layers. The respiratory epithelium lining the septum at Level I and the turbinates and septum at Level II was characterized by tall ciliated columnar cells interspersed with clusters of goblet cells, sometimes producing an irregular surface due to crowding of the cells (Plate 2). The severity of the respiratory hyperplasia was graded according to the extent of the surface area affected, the increase in the number of cell layers in the epithelium, and the increased height and cellular density of the respiratory epithelium.

Several exposure-related lesions were seen in the olfactory epithelium of Levels II and III. Basal cell hyperplasia of a minimal to mild degree was present in many of the 16 ppm animals and in most of the 32 and 64 ppm animals. The lesion was seen mainly along the nasal septum and the rounded tips of the turbinates of Level III and consisted of crowding of the basal cells along the basement membrane, increased numbers of basal cell layers, and occasional formation of small rosettes compressing the olfactory epithelium (Plate 3). Olfactory epithelial hyperplasia was seen in a few males and one female exposed to 64 ppm and was characterized by increased thickness of the entire epithelium. Hyperplasia of the underlying olfactory epithelium glands (Bowman's glands) was noted in a few 64 ppm males and 16 ppm females.

Mild to moderate olfactory epithelial atrophy, consisting of a decrease in the number of olfactory cells lining the turbinates and usually seen in the dorsal meatus of Level III, was present in the majority of 32 and 64 ppm males and females and in many of the 16 ppm males. Respiratory metaplasia of the olfactory epithelium occurred in many of the males in all exposed groups and in many of the 64 ppm females; metaplasia consisted of replacement of the normal olfactory epithelium by ciliated respiratory epithelium in the dorsal meatus of Level II and especially in the turbinates of Level III (Plate 4). The lesion was graded as mild to moderate on the basis of the extent of replacement of the normal olfactory epithelium.

Minimal to mild olfactory epithelial degeneration was diagnosed in a few 32 and 64 ppm males and females on the basis of either vacuolization of the epithelium or

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Nose in Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Male				
Number Examined Microscopically	49	49	50	49
Respiratory Epithelium, Hyperplasia[a]	5 (2.2)[b]	21** (1.6)	44** (1.9)	42** (2.5)
Glands, Respiratory Epithelium, Hyperplasia	3 (1.3)	14** (1.3)	39** (1.6)	45** (1.9)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	19** (1.0)	42** (1.4)	42** (1.7)
Olfactory Epithelium, Hyperplasia	0	1 (1.0)	3 (2.0)	5* (1.2)
Glands, Olfactory Epithelium, Hyperplasia	0	0	0	4* (1.8)
Olfactory Epithelium, Atrophy	1 (3.0)	21** (2.6)	26** (2.9)	26** (2.5)
Olfactory Epithelium, Metaplasia, Respiratory	1 (2.0)	10** (1.8)	18** (1.9)	29** (2.0)
Olfactory Epithelium, Degeneration	0	0	1 (1.0)	7** (1.3)
Olfactory Epithelium, Necrosis	0	0	2 (2.0)	6* (1.3)
Olfactory Epithelium, Accumulation, Hyaline Droplet	0	5* (1.0)	4 (1.3)	7** (1.6)
Inflammation, Chronic Active	9 (2.1)	12 (1.8)	22** (2.0)	28** (1.9)
Respiratory Epithelium, Adenoma[c]				
Overall rate[d]	0/49 (0%)	0/49 (0%)	0/50 (0%)	3/49 (6%)
Adjusted rate[e]	0.0%	0.0%	0.0%	7.9%
Terminal rate[f]	0/27 (0%)	0/23 (0%)	0/15 (0%)	1/16 (6%)
First incidence (days)	—[g]	—	—	661
Poly-3 test[h]	P=0.010	—[i]	—	P=0.102
Female				
Number Examined Microscopically	49	49	50	50
Respiratory Epithelium, Hyperplasia	2 (2.0)	23** (1.5)	25** (1.7)	36** (1.8)
Glands, Respiratory Epithelium, Hyperplasia	2 (1.5)	33** (1.2)	44** (1.5)	47** (1.5)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	28** (1.0)	42** (1.6)	48** (1.9)
Olfactory Epithelium, Hyperplasia	0	0	0	1 (3.0)
Glands, Olfactory Epithelium, Hyperplasia	0	6* (1.2)	1 (1.0)	2 (1.0)
Olfactory Epithelium, Atrophy	3 (2.3)	0	28** (2.8)	37** (2.9)
Olfactory Epithelium, Metaplasia, Respiratory	3 (1.7)	2 (2.0)	7 (1.4)	17** (1.8)
Olfactory Epithelium, Degeneration	0	0	1 (2.0)	4 (2.0)
Olfactory Epithelium, Necrosis	0	0	2 (2.0)	5* (1.6)
Olfactory Epithelium, Accumulation, Hyaline Droplet	6 (1.5)	5 (1.4)	6 (1.5)	15* (1.4)
Inflammation, Chronic Active	7 (1.6)	9 (1.7)	11 (1.4)	18* (1.6)
Glands, Carcinoma	0	1	0	0
Respiratory Epithelium, Adenoma	0	0	1	0

* Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year inhalation studies with chamber controls given NTP-2000 diet (mean ± standard deviation): 1/447 (0.2% ± 0.7%), range 0%-2%; all routes: 2/1,439 (0.1% ± 0.5%), range 0%-2%
[d] Number of animals with neoplasm per number of animals with nose examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Not applicable; no neoplasms in animal group
[h] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[i] Value of statistic cannot be computed.

an increase in foci of mineralization. Olfactory epithelial necrosis, graded as minimal to mild on the basis of extent, was also noted in a few 32 and 64 ppm males and females. Minimal to mild olfactory epithelial hyaline droplet accumulation was diagnosed in a few males and females in all exposed groups.

Chronic active inflammation was present in many of the exposed animals, as well as in some of the chamber controls. Lesion prevalence increased with exposure, although a concomitant increase in severity was not observed. The inflammation was characterized by infiltration of the lamina propria by lymphocytes, macrophages, and small numbers of neutrophils, and often by the presence of a suppurative exudate in the nasal cavity.

Mononuclear Cell Leukemia: The incidences of mononuclear cell leukemia in males occurred with a positive trend, and the incidence in the 64 ppm group was significantly greater than in the chamber control group; the incidence exceeded the NTP historical control ranges for inhalation studies and for all study routes (Tables 12, A2, and A3b).

The incidences of mononuclear cell leukemia in females increased with increasing exposure concentration, but none of the exposed groups had significantly greater incidences than the chamber controls, and the exposed group incidences were within the current NTP historical control ranges (Tables 12, B2, and B3a).

Mononuclear cell leukemia was staged using diagnostic criteria established by the National Toxicology Program (NTP, 2006). No significant differences were found in the average staging scores among exposed males (0 ppm, 2.8; 16 ppm, 2.8; 32 ppm, 2.7; 64 ppm, 2.9) or females (2.5, 2.5, 2.3, 2.6).

TABLE 12
Incidences of Mononuclear Cell Leukemia in Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Male				
All Organs[a]				
Overall rate[b]	21/49 (43%)	26/50 (52%)	23/50 (46%)	37/50 (74%)
Adjusted rate[c]	48.2%	57.6%	52.5%	80.1%
Terminal rate[d]	12/27 (44%)	11/23 (48%)	5/13 (33%)	11/16 (69%)
First incidence (days)	589	418	523	481
Poly-3 test[e]	P<0.001	P=0.246	P=0.424	P<0.001
Female				
All Organs[f]				
Overall rate	20/50 (40%)	13/50 (26%)	23/50 (46%)	26/50 (52%)
Adjusted rate	45.0%	28.4%	49.1%	56.0%
Terminal rate	11/29 (38%)	8/31 (26%)	11/27 (41%)	13/26 (50%)
First incidence (days)	513	516	439	502
Poly-3 test	P=0.040	P=0.074N	P=0.427	P=0.195

[a] Historical incidence for 2-year inhalation studies with chamber controls given NTP-2000 diet (mean ± standard deviation): 219/449 (48.8% ± 8.0%), range 38%-66%; all routes: 559/1,449 (38.5% ± 12.0%), range 18%-66%
[b] Number of animals with neoplasm per number of animals necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposed group is indicated by **N**.
[f] Historical incidence for inhalation studies: 156/450 (34.7% ± 11.7%), range 20%-52%; all routes: 342/1,350 (25.5% ± 10.9%), range 10%-52%

Preputial gland: Positive trends in the incidences of preputial gland adenoma (3/49, 0/49, 1/50, 6/49) and preputial gland adenoma or carcinoma (combined) (3/49, 0/49, 1/50, 7/49) were noted in males (Table A2). Although the incidences of these lesions in 64 ppm males were outside of the historical control ranges (adenoma: inhalation, 0%-6%; all routes, 0%-10%; adenoma or carcinoma (combined): inhalation, 0%-6%; all routes, 0%-12%; Table A3c), neither reached a level of statistical significance compared to the chamber control group, and there was no dose response. These lesions were not considered to be related to treatment.

MICE

2-WEEK STUDY

All mice exposed to 250 or 500 ppm died on day 1 of the study, and mice exposed to 125 ppm died on day 2 or 3 (Table 13). Final mean body weights and body weight gains of males and females exposed to 62.5 ppm were significantly less than those of the chamber controls. Abnormal breathing was observed on day 1 in all mice exposed to 125 ppm. After day 2 of exposure, eye discharge and lethargy were observed in one 125 ppm male, and lethargy was observed in two 125 ppm females. Abnormal breathing, lethargy, and thinness were observed in the 62.5 ppm males and females.

Absolute and relative right kidney weights of 31.3 ppm mice were significantly greater than those of the chamber controls (Table G3). Absolute and relative thymus weights of 62.5 ppm males were significantly less than for chamber controls. No exposure-related gross lesions were seen at necropsy, although many of the mice that died early had dark livers.

The livers of all males and females exposed to 250 or 500 ppm exhibited marked periportal necrosis, congestion, and erythrophagocytosis; these lesions also occurred

TABLE 13
Survival and Body Weights of Mice in the 2-Week Inhalation Study of Propargyl Alcohol

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	22.5 ± 0.6	26.7 ± 0.8	4.3 ± 0.3	
31.3	5/5	22.8 ± 0.6	26.6 ± 0.6	3.9 ± 0.4	100
62.5	5/5	22.3 ± 0.5	23.6 ± 0.3**	1.3 ± 0.5**	88
125	0/5[c]	21.9 ± 0.3	—	—	—
250	0/5[d]	22.5 ± 0.6	—	—	—
500	0/5[d]	22.4 ± 0.8	—	—	—
Female					
0	5/5	19.2 ± 0.4	22.6 ± 0.8	3.4 ± 0.6	
31.3	5/5	18.7 ± 0.2	22.6 ± 0.3	3.9 ± 0.3	100
62.5	5/5	18.7 ± 0.4	20.5 ± 0.5*	1.8 ± 0.3*	90
125	0/5[e]	18.6 ± 0.5	—	—	—
250	0/5[d]	18.8 ± 0.2	—	—	—
500	0/5[d]	18.6 ± 0.3	—	—	—

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Days of death: 2, 2, 2, 2, 3
[d] Day of deaths: 1
[e] Days of death: 2, 2, 2, 3, 3

in all 125 ppm males with less severity (Table 14). Many hepatocytes appeared to be distended by erythrocytes, and the nuclei were often condensed or missing. Other hepatocytes in the area were vacuolated. Hepatic lesions in the 125 ppm females were much less severe; all 125 ppm females had mild centrilobular necrosis, and a few 125 ppm females had minimal or mild erythrophagocytosis or congestion.

Exposure Concentration Selection Rationale: Based on the decreased survival of males and females exposed to 125 ppm or greater, the propargyl alcohol exposure concentrations selected for the 3-month inhalation study in mice were 4, 8, 16, 32, and 64 ppm. Although final mean body weights were decreased in males and females exposed to 62.5 ppm, decreased body weight gain occurred primarily during the first week of the study with recovery observed during the second week.

TABLE 14
Incidences of Nonneoplastic Lesions of the Liver in Mice in the 2-Week Inhalation Study of Propargyl Alcohol

	Chamber Control	31.3 ppm	62.5 ppm	125 ppm	250 ppm	500 ppm
Male						
Number Examined Microscopically	5	0	5	5	5	5
Hepatocytes, Necrosis, Periportal[a]	0		0	5** (3.4)[b]	5** (4.0)	5** (4.0)
Congestion, Periportal	0		0	5** (3.0)	5** (4.0)	5** (4.0)
Erythrophagocytosis, Periportal	0		0	5** (2.6)	5** (4.0)	5** (4.0)
Female						
Number Examined Microscopically	5	0	5	5	5	5
Hepatocytes, Necrosis, Periportal	0		0	0	5** (4.0)	5** (4.0)
Congestion, Periportal	0		0	1 (2.0)	5** (4.0)	5** (4.0)
Erythrophagocytosis, Periportal	0		0	3 (1.3)	5** (4.0)	5** (4.0)
Hepatocytes, Necrosis, Centrilobular	0		0	5** (2.0)	0	0

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

3-MONTH STUDY

All mice survived to the end of the study (Table 15). Final mean body weights and body weight gains of males exposed to 8 ppm or greater and 32 and 64 ppm females were significantly less than those of the chamber control groups. Abnormal breathing was observed in 64 ppm males and females on days 8 and 9.

Changes in hematology variables in males were not considered toxicologically relevant; no changes occurred in females (Table F2).

There were no biologically significant differences in organ weights between exposed and chamber control groups (Table G4). Reproductive tissue parameters of exposed males were similar to those of the chamber controls (Table H3). Only 2/9 female mice in the 64 ppm group exhibited regular estrous cyclicity compared to 6/10 in the controls (Table H4). Females exposed to 16 ppm differed from chamber controls in the relative time in the estrous stages, and 64 ppm females had a significantly increased probability of extended estrus ($p < 0.001$). No gross lesions were observed at necropsy.

Histopathologic changes were noted primarily in the nasal cavity, involving both the respiratory and olfactory epithelium of all three levels. The changes were limited to males and females exposed to 16 ppm or greater (Table 16).

Minimal to mild suppurative inflammation of the nasal cavity involving Level I and sometimes Level II occurred in many 64 ppm males, all 64 ppm females, and one 32 ppm female. Squamous metaplasia of the respiratory epithelium was present in most 32 ppm and all 64 ppm animals and was characterized by extensive involvement of the septum, turbinates, and lateral walls in nasal Level I. One 16 ppm female exhibited a single focus

TABLE 15
Survival and Body Weights of Mice in the 3-Month Inhalation Study of Propargyl Alcohol

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.6 ± 0.3	38.8 ± 0.8	15.2 ± 0.8	
4	10/10	23.4 ± 0.2	37.9 ± 0.4	14.4 ± 0.4	98
8	10/10	23.5 ± 0.3	36.4 ± 0.8*	12.9 ± 0.6**	94
16	10/10	23.5 ± 0.3	35.4 ± 1.0**	11.9 ± 0.8**	91
32	10/10	23.2 ± 0.3	34.5 ± 0.5**	11.3 ± 0.4**	89
64	10/10	23.3 ± 0.2	32.6 ± 0.5**	9.4 ± 0.4**	84
Female					
0	10/10	19.3 ± 0.2	30.9 ± 0.8	11.6 ± 0.7	
4	10/10	19.7 ± 0.3	31.1 ± 1.0	11.4 ± 0.8	109
8	10/10	19.1 ± 0.3	29.4 ± 0.9	10.3 ± 0.7	95
16	10/10	19.2 ± 0.3	30.2 ± 0.7	11.0 ± 0.4	98
32	10/10	19.1 ± 0.3	28.7 ± 0.5*	9.7 ± 0.5*	93
64	10/10	19.0 ± 0.2	27.5 ± 0.3**	8.5 ± 0.4**	89

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

TABLE 16
Incidences of Nonneoplastic Lesions of the Nose in Mice in the 3-Month Inhalation Study of Propargyl Alcohol

	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm	64 ppm
Male						
Number Examined Microscopically	9	10	10	10	10	10
Inflammation, Suppurative[a]	0	0	0	0	0	6** (1.2)[b]
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	6** (1.7)	10** (2.1)
Respiratory Epithelium, Degeneration, Hyaline	0	0	0	0	3 (1.0)	9** (1.9)
Olfactory Epithelium, Atrophy	0	0	0	0	8** (1.4)	10** (2.0)
Glands, Hyperplasia	0	0	0	4* (1.0)	9** (1.3)	10** (2.3)
Olfactory Epithelium, Necrosis	0	0	0	0	1 (1.0)	0
Turbinate, Atrophy	0	0	0	0	7** (1.7)	9** (2.0)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Inflammation, Suppurative	0	0	0	0	1 (2.0)	10** (1.9)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	1 (1.0)	7** (1.6)	10** (2.6)
Respiratory Epithelium, Degeneration, Hyaline	0	0	0	0	7** (1.9)	8** (1.8)
Olfactory Epithelium, Atrophy	0	0	0	0	7** (1.3)	10** (2.0)
Glands, Hyperplasia	0	0	0	1 (1.0)	8** (1.4)	10** (2.4)
Olfactory Epithelium, Necrosis	0	0	0	9** (1.0)	4* (1.0)	0
Turbinate, Atrophy	0	0	0	0	10** (2.3)	10** (3.0)

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

of minimal squamous metaplasia on the lateral wall. Minimal to mild hyaline degeneration (accumulation) was noted in the respiratory epithelium of nasal Level II in many of the 32 and 64 ppm males and females and manifested as brightly eosinophilic cytoplasmic globules in the epithelial cells lining the septum and dorsal meatus.

Minimal to mild olfactory epithelial atrophy involving Level II or III was present in most 32 and 64 ppm males and females and was characterized by decreased numbers of neuronal cells. The olfactory epithelium was often replaced by respiratory metaplastic epithelium. Hyperplasia of glands in the olfactory region involving the dorsal meatus in Level II and the dorsal meatus and septum in Level III was found in nearly all 32 and 64 ppm males and females, in a few 16 ppm males, and in one 16 ppm female. The glands were dilated, sometimes contained inflammatory cells and proteinaceous cell debris, and were often located beneath an overlying atrophic olfactory epithelium.

Necrosis of olfactory epithelium was noted in nasal Level II of most 16 ppm females, a few 32 ppm females, and one 32 ppm male. The necrosis was minimal in all cases and was characterized by disruption and sloughing of olfactory epithelium at the junction of respiratory and olfactory epithelium on the septum or adjacent nasoturbinate.

Turbinate atrophy was also present in all females and most males exposed to 32 or 64 ppm. The atrophy

ranged from minimal to moderate and was characterized by loss of the hook shape of the nasoturbinates and shortening of the maxilloturbinates in Level I and decrease in the length of the nasoturbinates in Level II. The change in shape and length of the turbinates was associated with, and largely due to, similar atrophic changes in the turbinate bone.

Exposure Concentration Selection Rationale: Based on significant reductions in final mean body weights of males and females exposed to 64 ppm, propargyl alcohol exposure concentrations selected for the 2-year inhalation study in mice were 8, 16, and 32 ppm. Males and females exposed to 64 ppm gained less weight than controls throughout the 3-month study.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 17 and in the Kaplan-Meier survival curves (Figure 5). Survival of exposed groups of mice was similar to that of the chamber control groups.

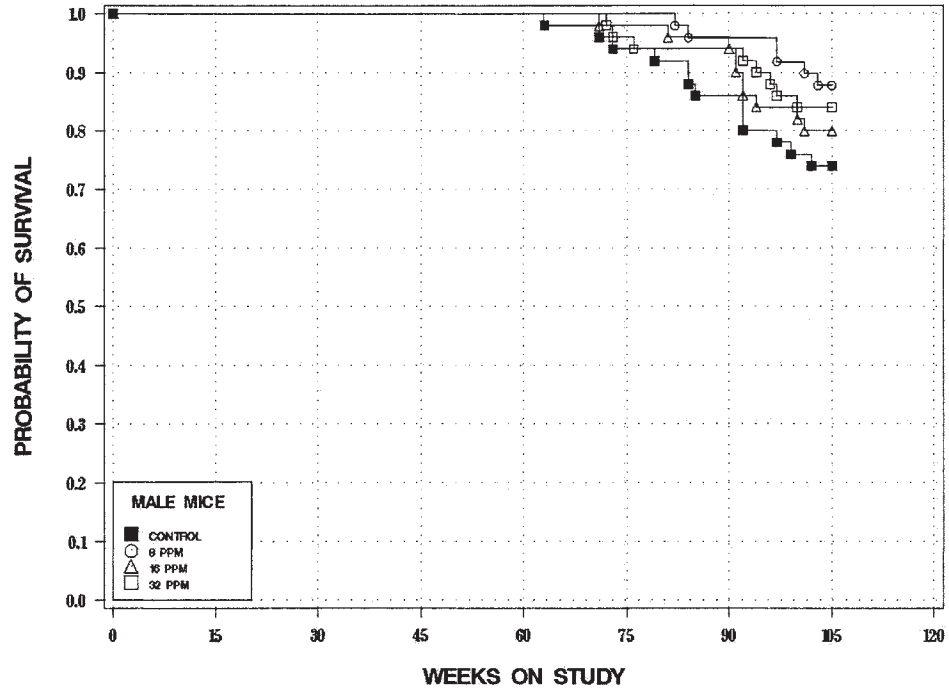
Body Weights and Clinical Findings

Mean body weights of 32 ppm males were less than those of the chamber control group during the first year of the study but recovered during the second year (Figure 6 and Table 18). Mean body weights of 16 and 32 ppm females were less than those of the chamber controls after weeks 73 and 21, respectively (Figure 6 and Table 19). Eye abnormality was observed after 1 full year of exposure with the incidence increasing in exposed groups in an exposure concentration-related manner. There were 20% and 14% incidences of eye abnormality (unspecified) in 32 ppm males and females, respectively, compared to incidences of 8% and 2% in chamber control males and females, respectively.

TABLE 17
Survival of Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	1	0	0
Moribund	9	6	6	7
Natural deaths	4	0	4	1
Animals surviving to study termination	37	43[b]	40	42
Percent probability of survival at end of study[c]	74	88	80	84
Mean survival (days)[d]	694	706	709	710
Survival analysis[e]	P=0.453N	P=0.113N	P=0.583N	P=0.303N
Female				
Animals initially in study	50	50	50	50
Moribund	9	8	15	10
Natural deaths	2	3	3	2
Animals surviving to study termination	39	39	32	38
Percent probability of survival at end of study	78	78	64	76
Mean survival (days)	699	708	688	674
Survival analysis	P=0.680	P=1.000N	P=0.184	P=0.932

[a] Censored from survival analysis
[b] Includes one animal that died during the last week of the study.
[c] Kaplan-Meier determinations
[d] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[e] The result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by **N**.



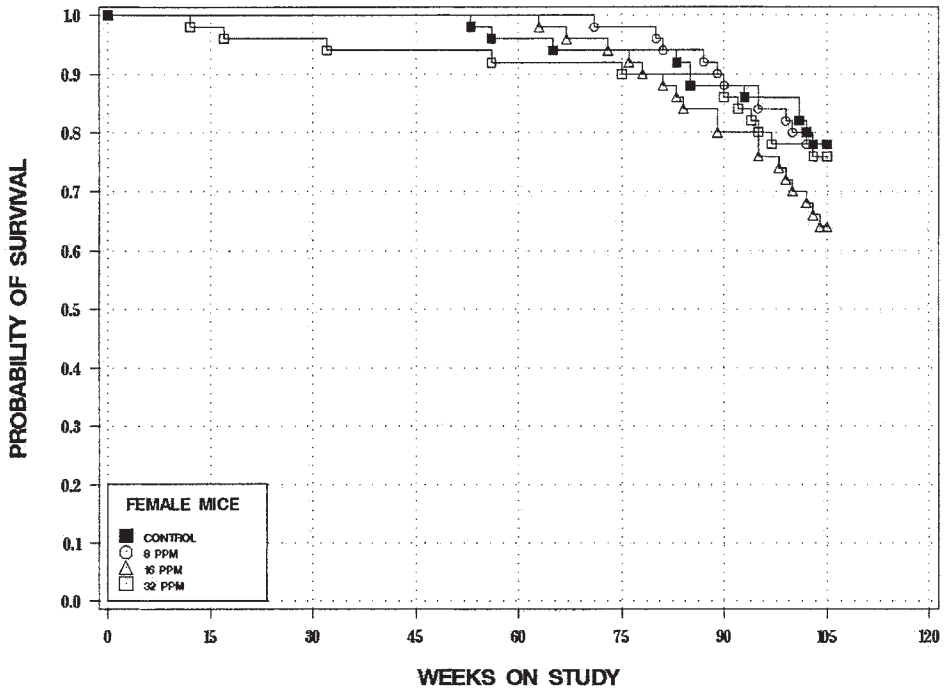


FIGURE 5
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Propargyl Alcohol by Inhalation for 2 Years

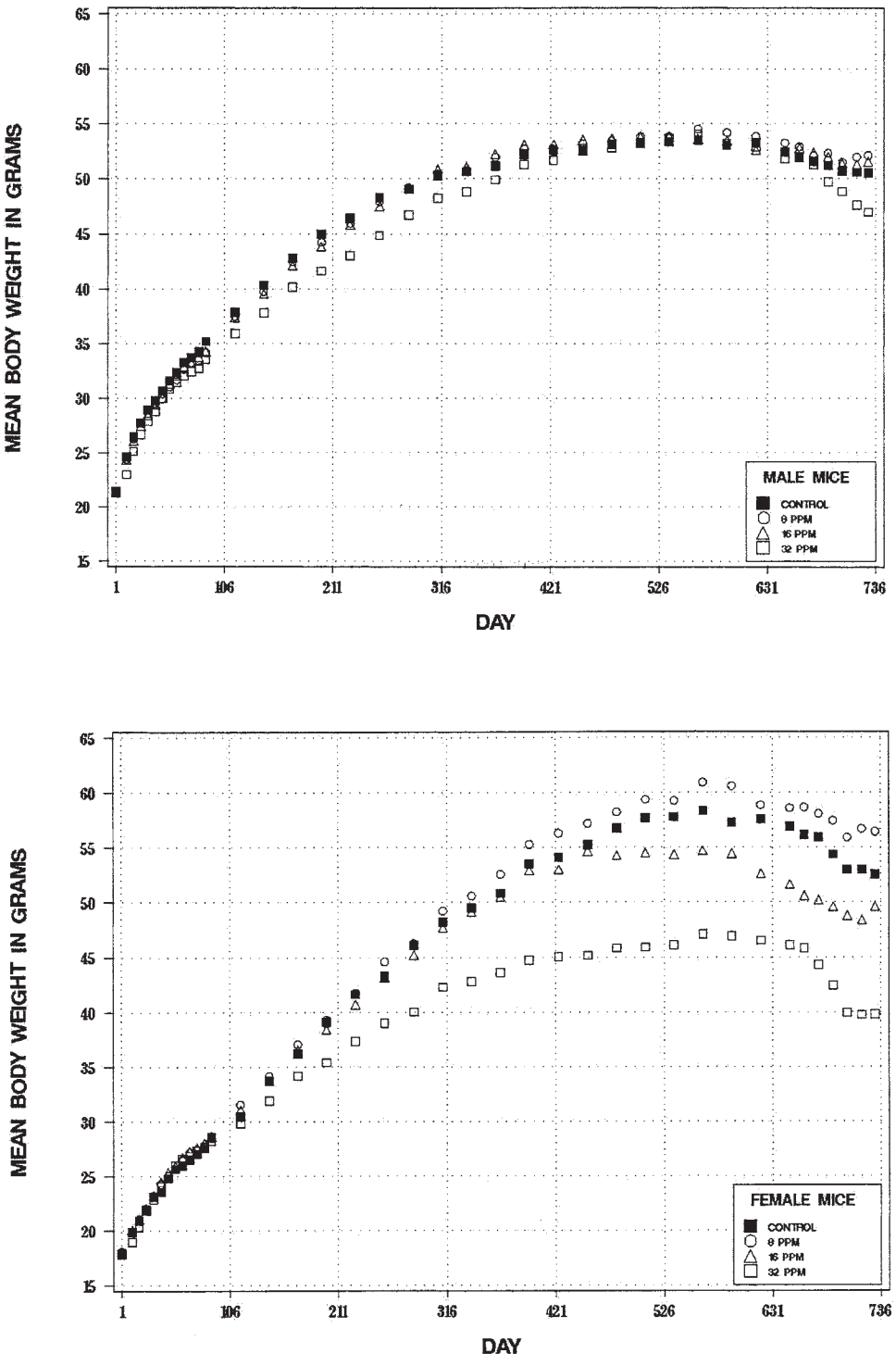


FIGURE 6
Growth Curves for Male and Female Mice
Exposed to Propargyl Alcohol by Inhalation for 2 Years

TABLE 18
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol

Days on Study	Chamber Control		8 ppm			16 ppm			32 ppm		
	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	21.7	50	21.3	99	50	21.4	99	50	21.3	99	50
11	24.6	50	24.4	99	50	24.3	99	50	23.0	94	50
18	26.4	50	26.3	99	50	26.1	99	50	25.1	95	50
25	27.7	50	27.7	100	50	27.5	99	50	26.7	96	50
32	29.0	50	28.6	99	50	28.5	98	50	27.9	96	50
39	29.8	50	29.5	99	50	29.4	99	50	28.8	97	50
46	30.7	50	29.9	98	49	30.4	99	50	30.0	98	50
53	31.6	50	31.0	98	49	31.3	99	50	30.8	98	50
60	32.3	50	31.7	98	49	32.0	99	50	31.4	97	50
67	33.2	50	32.6	98	49	32.9	99	50	32.0	96	50
74	33.7	50	33.2	99	49	33.2	99	50	32.4	96	50
81	34.2	50	33.4	98	49	33.7	99	50	32.7	95	50
88	35.2	50	34.1	97	49	34.3	98	50	33.6	95	50
116	37.8	50	37.5	99	49	37.4	99	50	35.9	95	50
144	40.3	50	39.7	99	49	39.5	98	50	37.8	94	50
172	42.8	50	42.5	99	49	42.1	99	50	40.2	94	50
200	45.0	50	44.2	98	49	43.8	98	50	41.6	93	50
228	46.4	50	45.9	99	49	45.8	99	50	43.0	93	50
256	48.3	50	47.9	99	49	47.5	98	50	44.9	93	50
284	49.0	50	49.2	100	49	49.2	100	50	46.7	95	50
312	50.3	50	50.4	100	49	50.9	101	50	48.2	96	50
340	50.7	50	50.7	100	49	51.1	101	50	48.8	96	50
368	51.2	50	52.0	102	49	52.3	102	50	49.9	98	50
396	52.2	50	52.7	101	49	53.1	102	50	51.3	98	50
424	52.6	50	52.7	100	49	53.1	101	50	51.7	98	50
452	52.6	49	53.2	101	49	53.6	102	50	52.5	100	50
480	53.1	49	53.4	101	49	53.7	101	50	52.8	99	50
508	53.3	48	53.8	101	49	53.8	101	49	53.2	100	48
536	53.3	47	53.8	101	49	53.9	101	49	53.7	101	47
564	53.5	46	54.5	102	49	53.6	100	49	54.0	101	47
592	53.0	44	54.2	102	47	53.5	101	48	53.2	100	47
620	53.3	43	53.8	101	47	52.6	99	48	52.9	99	47
648	52.4	40	53.3	102	47	52.5	100	43	51.8	99	46
662	51.9	40	52.9	102	47	52.8	102	42	51.9	100	45
676	51.6	40	52.1	101	47	52.4	102	42	51.3	99	44
690	51.2	38	52.3	102	45	52.0	101	42	49.7	97	43
704	50.7	38	52.0	103	44	51.4	101	41	48.9	96	42
718	50.6	37	52.0	103	44	51.3	101	40	47.6	94	42
Mean for weeks											
1-13	30.0		29.5	99		29.6	99		28.9	96	
14-52	45.6		45.3	99		45.3	99		43.0	94	
53-103	52.3		53.0	102		52.9	101		51.7	99	

TABLE 19
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol

Days on Study	Chamber Control		8 ppm			16 ppm			32 ppm		
	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	18.0	50	18.1	101	50	18.1	101	50	17.9	100	50
11	19.8	50	19.9	100	50	20.1	101	50	18.9	95	50
18	20.9	50	21.0	100	50	21.0	100	50	20.3	97	50
25	21.9	50	22.0	101	50	22.0	101	50	21.8	100	50
32	23.1	50	23.2	101	50	23.3	101	50	22.9	99	50
39	23.6	50	24.3	103	50	24.5	104	50	24.1	102	50
46	24.8	50	25.0	101	50	25.3	102	50	24.8	100	50
53	25.7	50	25.7	100	50	25.9	101	50	26.0	101	50
60	26.0	50	26.4	102	50	26.7	103	50	26.6	102	50
67	26.5	50	27.2	103	50	27.3	103	50	26.9	101	50
74	27.0	50	27.5	102	50	27.6	102	50	27.4	101	50
81	27.5	50	27.8	101	50	28.0	102	50	27.7	101	49
88	28.5	50	28.5	100	50	28.7	101	50	28.3	99	49
116	30.4	50	31.5	104	50	31.1	102	50	29.8	98	49
144	33.7	50	34.1	101	50	33.7	100	50	31.9	95	48
172	36.2	50	37.0	102	50	36.5	101	50	34.1	94	48
200	39.1	50	39.3	101	50	38.4	98	50	35.4	91	48
228	41.6	50	41.7	100	50	40.7	98	50	37.3	90	47
256	43.3	50	44.6	103	50	43.2	100	50	39.0	90	47
284	46.1	50	46.3	100	50	45.2	98	50	40.0	87	47
312	48.2	50	49.2	102	50	47.7	99	50	42.3	88	47
340	49.5	50	50.6	102	50	49.1	99	50	42.8	86	47
368	50.8	50	52.6	103	50	50.5	99	50	43.6	86	47
396	53.5	48	55.3	103	50	52.9	99	50	44.7	84	46
424	54.1	48	56.3	104	50	53.0	98	50	45.1	83	46
452	55.3	48	57.2	104	50	54.7	99	49	45.2	82	46
480	56.8	47	58.2	103	50	54.3	96	48	45.8	81	46
508	57.7	47	59.3	103	49	54.5	95	48	45.9	80	46
536	57.8	47	59.2	103	49	54.4	94	46	46.1	80	45
564	58.3	47	60.9	105	48	54.7	94	45	47.1	81	45
592	58.0	45	60.6	105	47	54.4	94	42	46.9	81	45
620	57.6	44	58.9	102	45	52.7	92	40	46.5	81	45
648	56.9	43	58.6	103	44	51.6	91	40	46.1	81	42
662	56.2	43	58.6	104	42	50.6	90	39	45.8	82	41
676	56.0	43	58.1	104	42	50.2	90	38	44.3	79	40
690	54.4	43	57.4	106	41	49.6	91	37	42.4	78	39
704	53.1	41	55.9	105	40	48.8	92	35	39.9	75	39
718	53.0	39	56.7	107	39	49.0	93	33	40.0	75	38
Mean for weeks											
1-13	24.1		24.4	101		24.5	102		24.1	100	
14-52	40.9		41.6	102		40.6	99		37.0	91	
53-103	55.6		57.7	104		52.2	94		44.7	81	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the nose, Harderian gland, and eye. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix C for male mice and Appendix D for female mice, and historical incidences for Harderian gland adenoma are presented in Appendix C for male mice.

Nose: Nasal respiratory epithelial adenomas occurred in a few animals in each exposed group; the incidences increased in an exposure concentration-related manner, and the incidence was significantly increased in the 32 ppm males and females (Tables 20, C2, and D2). No adenomas occurred in chamber controls, and none were reported in historical control mice (males: inhalation 0/449, all routes 0/1,492; females: inhalation 0/447, all routes 0/1,593). The adenomas originated from the nasoturbinates and lateral walls of Levels I and II, forming sessile or pedunculated polypoid masses projecting into the nasal cavity (Plate 5). Microscopically, the adenomas were characterized by proliferation of well-differentiated, cuboidal to columnar transitional or respiratory epithelium. Some of the adenomas were relatively solid with interspersed gland-like structures, while other adenomas exhibited a peripheral rim of epithelium surrounding a central cystic fibrovascular stroma (Plate 6).

A spectrum of nonneoplastic changes was observed in the respiratory and olfactory epithelium at all exposure concentrations (Tables 20, C4, and D3). Respiratory epithelial hyperplasia, usually mild to moderate, occurred in most mice exposed to propargyl alcohol. The severity increased only minimally with exposure concentration. Hyperplasia involved the tips of the nasoturbinates and their lateral sides, the lateral walls, and the maxilloturbinates in nasal Level I; the septum was occasionally involved. Level II was less often and less extensively affected. Hyperplasia was characterized by thickening of the respiratory epithelium due to increased numbers of columnar and cuboidal lining cells. Respiratory glandular hyperplasia was also seen in most 16 and 32 ppm mice and in many 8 ppm mice, with exposure concentration-related increases in incidence and severity. The glandular hyperplasia primarily involved the mucosa of the dorsal meatus of Levels I and II (Plate 7).

Squamous metaplasia of the respiratory epithelium occurred in most 16 and 32 ppm males and females and several 8 ppm males, with exposure concentration-related increases in incidence and severity. The metaplasia was noted predominantly in Level I and, to a lesser extent, Level II. The extent of the metaplasia varied from a few foci on turbinate tips to nearly the entire nasal mucosa in Level I. Suppurative inflammation was often associated with the squamous metaplasia, presumably preceding it in response to injury of the nose. The inflammatory response consisted of a submucosal, mucosal, or luminal infiltration by neutrophils with occasional disruption of the surface epithelium and accumulation of inflammatory exudate and proteinaceous debris in the nasal airways.

Turbinate atrophy with an exposure concentration-related increase in severity was found in most exposed males and all exposed females. Atrophy was most apparent in Level I and consisted of a loss of the normal hook shape of the nasoturbinates and maxilloturbinates. The tips of the atrophic nasoturbinates frequently exhibited either hyperplasia or squamous metaplasia of the respiratory epithelium, as well as occasional respiratory adenomas.

Olfactory epithelial atrophy occurred in many 16 and 32 ppm males and females. This lesion also occurred in two female chamber controls and a few mice exposed to 8 ppm. An exposure concentration-related increase in severity was noted. The designation of atrophy was restricted to lesions showing a decrease in the number of layers of olfactory cells and thus a decreased height of the olfactory epithelium; the lesion was usually present in the dorsal meatus of Level III (Plate 8). When the olfactory epithelium was replaced by ciliated, columnar respiratory epithelium, the alteration was designated respiratory metaplasia. This latter lesion was noted with increased incidence in several of the 32 ppm males and females; it also occurred in a few 16 ppm mice, some of the male chamber controls, and one female chamber control. Increasing severity was represented by more extensive replacement of the olfactory epithelium by the respiratory metaplastic epithelium, which was frequently accompanied by underlying respiratory glandular metaplasia and atrophy of the olfactory nerve bundles.

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Nose in Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Male				
Number Examined Microscopically	49	50	50	50
Respiratory Epithelium, Hyperplasia[a]	1 (1.0)[b]	49** (2.0)	49** (2.4)	50** (2.3)
Glands, Respiratory Epithelium, Hyperplasia	17 (1.0)	29* (1.6)	40** (1.8)	50** (2.2)
Respiratory Epithelium, Metaplasia, Squamous	2 (1.0)	11* (1.0)	36** (1.3)	50** (2.4)
Inflammation, Suppurative	2 (1.0)	16** (1.0)	25** (1.2)	50** (1.9)
Turbinate, Atrophy	0	50** (1.5)	49** (2.6)	50** (3.2)
Olfactory Epithelium, Atrophy	0	3 (1.0)	21** (1.2)	33** (2.2)
Olfactory Epithelium, Metaplasia, Respiratory	5 (1.2)	0*	7 (1.3)	16* (1.8)
Respiratory Epithelial Adenoma[c]				
Overall rate[d]	0/49 (0%)	1/50 (2%)	4/50 (8%)	7/50 (14%)
Adjusted rate[e]	0.0%	2.1%	8.6%	14.9%
Terminal rate[f]	0/37 (0%)	1/43 (2%)	3/40 (8%)	7/42 (17%)
First incidence (days)	—[g]	729 (T)	698	729 (T)
Poly-3 test[h]	P<0.001	P=0.517	P=0.069	P=0.010
Female				
Number Examined Microscopically	50	50	50	50
Respiratory Epithelium, Hyperplasia	0	50** (1.9)	50** (2.2)	49** (2.2)
Glands, Respiratory Epithelium, Hyperplasia	7 (1.1)	24** (1.2)	44** (1.7)	49** (2.1)
Respiratory Epithelium, Metaplasia, Squamous	0	3 (1.0)	34** (1.5)	49** (2.4)
Inflammation, Suppurative	1 (1.0)	4 (1.0)	35** (1.2)	45** (2.0)
Turbinate, Atrophy	0	50** (1.5)	50** (2.7)	50** (3.5)
Olfactory Epithelium, Atrophy	2 (1.0)	5 (1.2)	31** (1.7)	29** (2.2)
Olfactory Epithelium, Metaplasia, Respiratory	1 (1.0)	0	6* (1.7)	14** (1.9)
Respiratory Epithelial Adenoma[i]				
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)	6/50 (12%)
Adjusted rate	0.0%	4.3%	9.3%	13.7%
Terminal rate	0/39 (0%)	2/39 (5%)	3/32 (9%)	6/38 (16%)
First incidence (days)	—	731 (T)	722	731 (T)
Poly-3 test	P=0.006	P=0.241	P=0.054	P=0.013

(T) Terminal sacrifice
* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test.
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year inhalation studies with chamber controls given NTP-2000 diet 0/449; all routes 0/1,492
[d] Number of animals with neoplasm per number of animals with nose examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Not applicable; no neoplasms in animal group
[h] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[i] Historical incidence for 2-year inhalation studies 0/447; all routes 0/1,593

Harderian gland: Significantly increased incidences of Harderian gland adenoma were noted in 8 and 32 ppm males (0 ppm, 3/50; 8 ppm, 10/50; 16 ppm, 6/50; 32 ppm, 11/50; Table C2). The incidence of adenoma in the 32 ppm males slightly exceeded the historical control range for inhalation studies [58/450 (13% ± 4%), range 6%-20%] and was at the upper bound of the range for all routes of administration [185/1,499 (13% ± 4%), range 4%-22%] (Table C3). The adenomas presented as circumscribed, unencapsulated nodules composed of tall columnar cells with abundant cytoplasm and basal nuclei arranged in tubular and papillary patterns. No statistically significant exposure concentration-related increases in Harderian gland carcinoma or Harderian gland adenoma or carcinoma (combined) were noted. The incidences were not increased in females.

Eye: Chronic active inflammation of the cornea occurred with increased incidence in 32 ppm males and females (Tables 21, C4, and D3). A few males and females exposed to lower concentrations also exhibited inflammation, but severities were similar across groups. Cataracts occurred with increased incidence in 32 ppm females, occurring in six females compared to one female in the 8 ppm group and one in the chamber control group. The severity did not increase with exposure concentration.

GENETIC TOXICOLOGY

Propargyl alcohol (3 to 10,000 µg/plate) was mutagenic in *Salmonella typhimurium* strain TA100 in the absence of liver S9 activation enzymes only; no mutagenicity was observed in TA100 in the presence of liver S9 enzymes (Table E1). In addition, propargyl alcohol was not mutagenic in *S. typhimurium* strain TA1535 without S9 or in TA98 with or without S9. *In vivo*, no significant increases in micronucleated normochromatic erythrocytes were seen in peripheral blood of male B6C3F1 mice exposed to 4, 8, 16, 32, or 64 ppm for 3 months by inhalation (Table E2). All groups of exposed male mice had micronucleated erythrocyte frequencies that were higher than the frequency in the chamber control group, but none of the increases reached statistical significance (P=0.005); level of response was not related to dose. In the female mice, the trend test was significant (P=0.002), but none of the individual exposed groups had micronucleated erythrocyte frequencies that were significantly increased over the chamber control group, and therefore, the test in female mice was judged to be equivocal. No significant changes in the percentage of polychromatic erythrocytes were observed in either male or female mice over the exposure range tested, indicating an absence of treatment-related toxicity to the bone marrow.

TABLE 21
Incidences of Nonneoplastic Lesions of the Eye in Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control		8 ppm		16 ppm		32 ppm	
Male								
Number Examined Microscopically	49		50		48		49	
Cornea, Inflammation, Chronic Active[a]	1	(4.0)[b]	0		5	(2.8)	7*	(2.6)
Cataract	0		0		1	(2.0)	2	(2.0)
Female								
Number Examined Microscopically	49		48		49		48	
Cornea, Inflammation, Chronic Active	0		1	(3.0)	2	(2.5)	10**	(2.0)
Cataract	1	(2.0)	1	(3.0)	0		6*	(1.8)

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test.
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

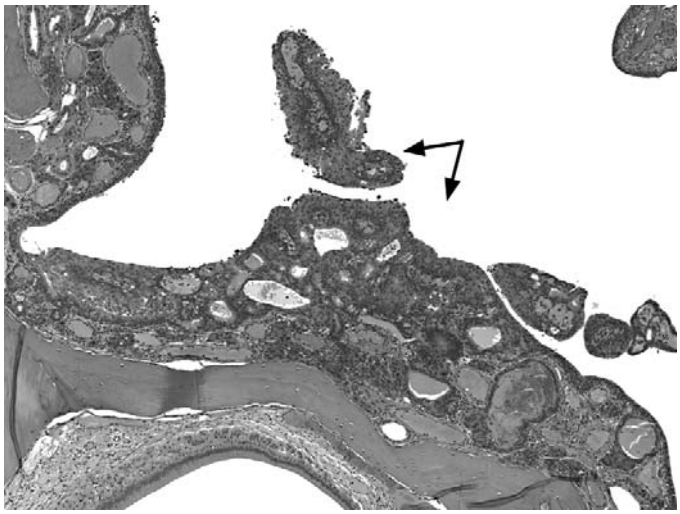


PLATE 1
Respiratory epithelial adenoma (arrows) arising from the lateral wall of the nasal cavity (Level I) of a male F344/N rat exposed by inhalation to 64 ppm propargyl alcohol for 2 years. H&E.

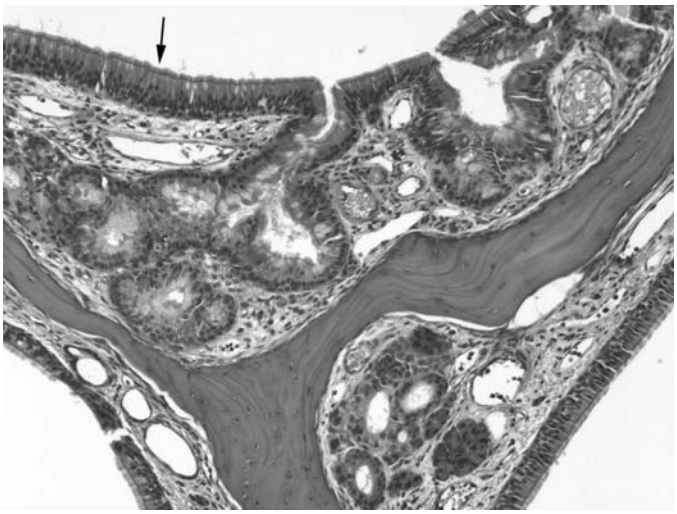


PLATE 2
Respiratory glandular hyperplasia (top half of plate) of nasal turbinate (Level II) contrasted with normal-sized respiratory glands (bottom half of plate) in a male F344/N rat exposed to 64 ppm propargyl alcohol by inhalation for 2 years. Note that the respiratory epithelium adjacent to the hyperplastic glands is also mildly hyperplastic (arrow). H&E.

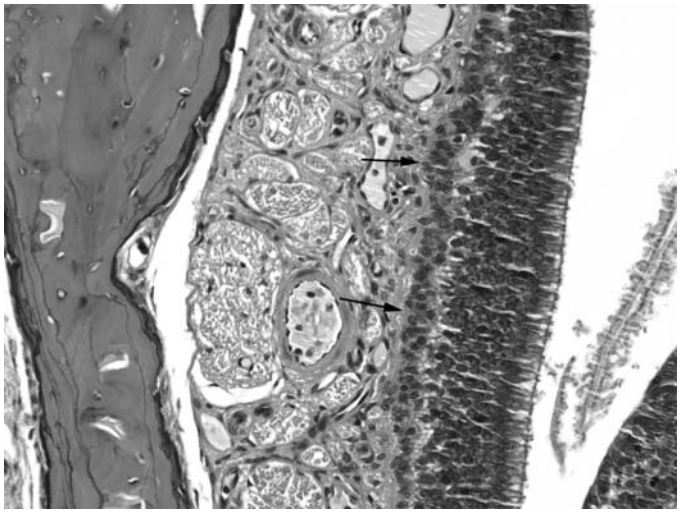


PLATE 3
Basal cell hyperplasia (arrows) consisting of 2 or 3 layers of basal cells rather than the usual single layer in the olfactory epithelium of the nasal cavity (Level III) of a male F344/N rat exposed to 64 ppm propargyl alcohol by inhalation for 2 years. H&E.

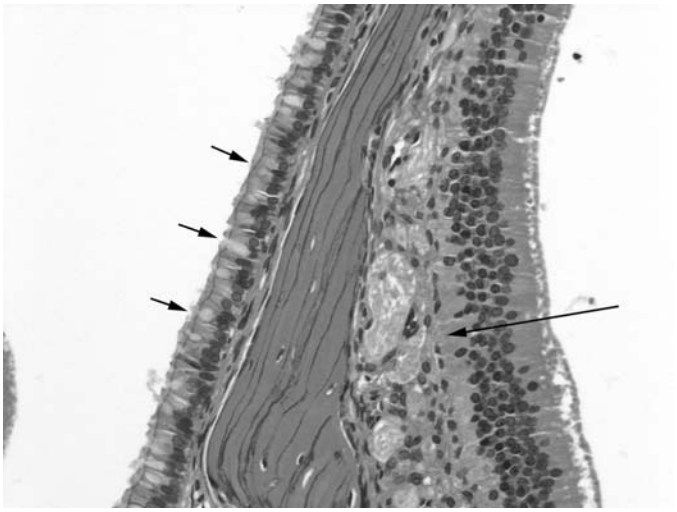


PLATE 4
Respiratory epithelial metaplasia (short arrows) replacing the olfactory epithelium on an ethmoid turbinate of the nasal cavity (Level III) in a female F344/N rat exposed by inhalation to 32 ppm propargyl alcohol for 2 years. On the opposite side of the turbinate, the olfactory epithelium exhibits hyaline accumulation in the basal portion of the epithelium (long arrow). Compare the multilayered olfactory epithelium (right) to the simple columnar ciliated epithelium with goblet cells characterizing the respiratory metaplastic epithelium (left). H&E.

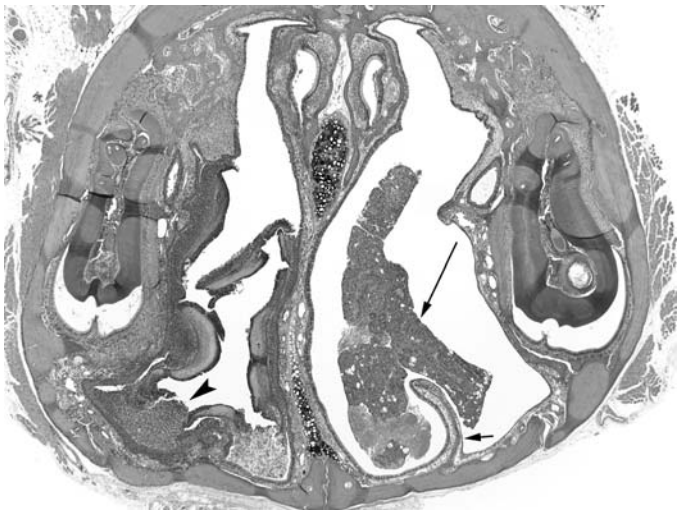


PLATE 5
Respiratory epithelial adenoma (long arrow) projecting into and partially occluding one side of the nasal cavity (Level I) in a male B6C3F1 mouse exposed to 32 ppm propargyl alcohol by inhalation for 2 years. Turbinate atrophy (short arrow) and suppurative inflammation on contralateral side (arrowhead) are also present. H&E.

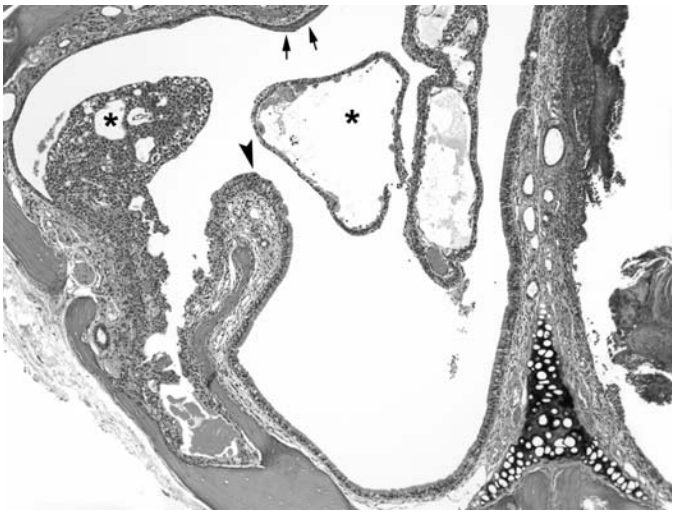


PLATE 6
Respiratory epithelial adenoma with solid and cystic components (asterisks) in the nasal cavity (Level I) of a male B6C3F1 mouse exposed by inhalation to 32 ppm propargyl alcohol for 2 years. Respiratory epithelial hyperplasia (arrowhead) with underlying turbinate atrophy and squamous metaplasia (short arrows) are also shown. Suppurative inflammation may be seen on the contralateral side of the nasal septum. H&E.

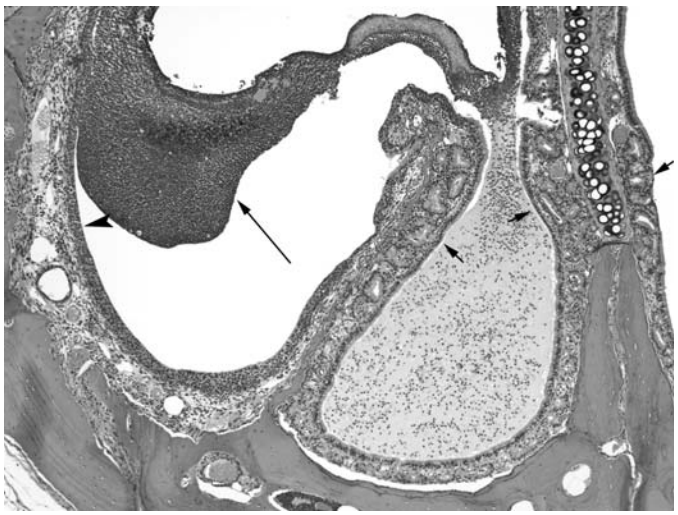


PLATE 7
Respiratory glandular hyperplasia (short arrows) of the nasal turbinate and nasal septum (Level II) in a male B6C3F1 mouse exposed by inhalation to 32 ppm propargyl alcohol for 2 years. Suppurative inflammatory exudate is present in the nasal cavity (long arrow). Squamous metaplastic epithelium partially replaces the respiratory epithelium on the lateral wall (arrowhead). H&E.

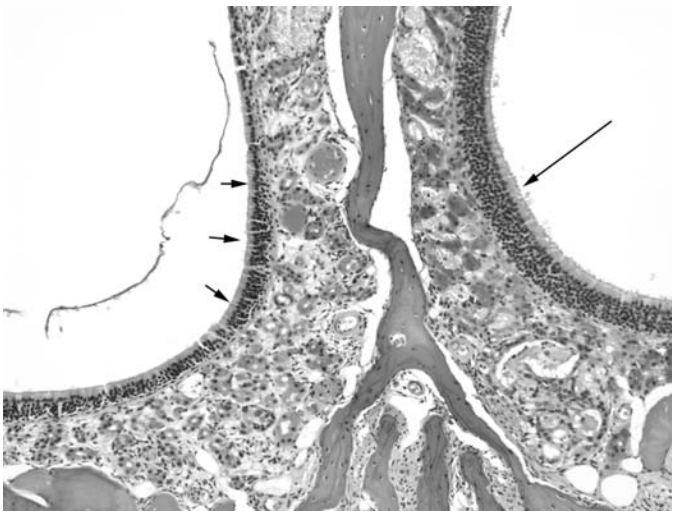


PLATE 8
Olfactory epithelial atrophy (short arrows) on one side of the nasal septum (Level III) in a male B6C3F1 mouse exposed to 32 ppm propargyl alcohol by inhalation for 2 years. Compare with olfactory epithelium of normal thickness (long arrow) on opposite side of the septum. H&E.

DISCUSSION AND CONCLUSIONS

Propargyl alcohol is used as a reactant/chemical intermediate, pharmaceutical intermediate, agricultural chemical intermediate, soil fumigant, corrosion inhibitor, solvent stabilizer, and polymer modifier. Toxicity and carcinogenicity studies of propargyl alcohol were performed because of its high production volume, potential for occupational exposure, and lack of chronic toxicity data. The current recommended threshold limit value for propargyl alcohol is 1 ppm (ACGIH, 2005).

In the 2-week studies, exposure to 125 ppm propargyl alcohol or greater resulted in significant mortality to rats and mice. Exposure concentrations for the 2-week studies were selected after review of a 2-week study conducted by industry using 200 ppm as the highest exposure concentration (BUA, 1998). Since only one female exposed to 200 ppm died in the industry study, decreased survival at 125 ppm in the current study was not expected. In addition, absorption, distribution, metabolism, and excretion studies conducted by the NTP indicated that only 20% to 30% of a 100 ppm exposure concentration was absorbed by male rats and mice during a 6-hour nose-only inhalation exposure; 1 and 10 ppm exposure concentrations were absorbed at about twice this level (Dix *et al.*, 2001).

A sharp dose response was observed for hepatotoxicity in rats and mice in the 2-week studies. Microscopic hepatic lesions including moderate to marked periportal necrosis, congestion, and hepatocellular erythrophagocytosis were observed in rats and mice exposed to 250 ppm and male mice exposed to 125 ppm. Male rats and female mice exposed to 125 ppm exhibited centrilobular necrosis. Administration of allyl alcohol (H_2C=$CHCH_2OH$), a structurally related unsaturated three-carbon alcohol, causes necrosis in periportal regions of the liver lobule in rodents (Badr, 1991). The ultimate toxicant is the reactive metabolite, acrolein, formed by the oxidation of allyl alcohol by alcohol dehydrogenase. It is proposed that higher oxygen tension in periportal regions plays an important role in the mechanism of localized allyl alcohol hepatotoxicity. Allyl alcohol has not been tested for carcinogenicity in chronic animal inhalation studies; however, it was not tumorigenic in male or female F344 rats or male Syrian hamsters in a 2-year oral bioassay (Lijinsky and Reuber, 1987). The toxicity of propargyl alcohol is likely due to metabolism to the more reactive and mutagenic aldehyde, propargylaldehyde (Basu and Marnett, 1984). Each molecule of propargylaldehyde can react with two molecules of glutathione possibly depleting glutathione more rapidly than allyl alcohol. Propargyl alcohol is a relatively poor substrate for alcohol dehydrogenase (DeMaster *et al*., 1994) and most of the biotransformation to its aldehyde is mediated by CYP2E1 (Moridani *et al*., 2001).

Liver and kidney toxicity as reported at higher exposure concentrations in other subchronic inhalation studies of propargyl alcohol (Dow Chemical, 1964; Lington and Bevan, 1994; BUA, 1998) were not observed in the current 3-month studies. This result was somewhat surprising given the structural similarity of propargyl alcohol to allyl alcohol. However, a threshold for hepatotoxicity was evident in the 2-week studies below which chronic hepatic effects were not observed. Although liver and kidney weights were increased in the 2-week studies, there was only a slight increase in liver and kidney weights of rats in the 3-month study. However, there was an indication of hepatic dysfunction based on the clinical pathology results. Total serum cholinesterase activity in rats was decreased in an exposure concentration-related manner at terminal sacrifice, particularly in females. To determine the nature of the serum cholinesterase inhibition, samples were analyzed for inhibition of butyrylcholinesterase activity; the butyrylcholinesterase fraction of the total cholinesterase activity appeared to be inhibited following propargyl alcohol exposure (data not shown). The mechanism for the decreases in cholinesterase activity is not known. Because butyrylcholinesterase is synthesized in the liver, a decrease in serum total cholinesterase activity that appears to be mostly related to decreases in the butyrylcholinesterase activity could suggest either selective inhibition of this cholinesterase or reduced production of the enzyme. The parent compound (propargyl alcohol) had no direct inhibitory effect. Thus, if enzyme inhibition were the mechanism, it presumably would have been related to a

metabolite of propargyl alcohol. Reduced cholinesterase production by the liver has been used as an indicator of hepatic insufficiency; however, in this study, there were no alterations in other markers of hepatic injury or in liver histopathology. Because female rats have a higher butyrylcholinesterase:acetylcholinesterase ratio (70:30 versus 50:50 in male rats; Dr. Stephanie Padilla, personal communication) in their serum, it would be consistent for the female rats to be more affected than the males when butyrylcholinesterase activity is affected.

The nose was the primary target organ in the 3-month inhalation studies of propargyl alcohol. In rats, the incidences of hyperplasia of the respiratory epithelium were significantly increased in all exposed groups except 8 ppm males and 4 ppm females. The incidences of necrosis of the olfactory epithelium were significantly increased in 64 ppm males and females, and squamous metaplasia of the respiratory epithelium occurred in most females exposed to 64 ppm. In mice, lesions included suppurative inflammation, squamous metaplasia of the respiratory epithelium, hyaline degeneration (accumulation) of the respiratory epithelium, olfactory epithelial atrophy, hyperplasia of glands in the olfactory region, necrosis of olfactory epithelium, and turbinate atrophy. These lesions occurred predominantly in the two highest exposure concentration groups (32 and 64 ppm). The no-observed-adverse-effect level in mice was 8 ppm.

The evaluation of vaginal smears revealed a decrease in the number of female mice in the 64 ppm group that exhibited regular estrous cycles compared to the chamber control group. Based on the SMVCE results, the reproductive organ weights, and histopathology of the reproductive system, there was no evidence of toxicity to the reproductive system of rats or male mice in this 3-month study. The evaluation of vaginal smears of 64 ppm females also had a significantly increased probability of extended estrus. Based on these data, propargyl alcohol has the potential to produce adverse effects in studies of fertility and reproductive performance.

In the 2-year rat study, survival of 32 and 64 ppm males was significantly reduced compared to chamber controls. The decreased survival was mostly a result of moribund sacrifice of animals with a total of 26 and 30 in the 32 and 64 ppm groups, respectively. Almost all of the moribund sacrifices were attributed to excessive lethargy.

As in the 3-month studies, the nose was the primary target organ in the 2-year inhalation studies of propargyl alcohol. A spectrum of nonneoplastic nasal lesions was observed in the respiratory and olfactory epithelium at all exposure concentrations in the 2-year studies (16 ppm or greater in rats, 8 ppm or greater in mice). A no-observed-adverse-effect level was not identified in the 2-year studies. The incidences of hyperplasia of the respiratory epithelium and underlying glands were significantly increased in male and female rats and mice at all exposure concentrations. Squamous metaplasia of the respiratory epithelium occurred in most male and female mice exposed to 16 or 32 ppm and was accompanied by suppurative inflammation. The incidences of atrophy and respiratory metaplasia in the olfactory epithelium were generally significantly increased in male and female rats and mice at the two highest exposure concentrations. A wider spectrum of nonneoplastic lesions of the olfactory epithelium was observed in rats compared to mice and included hyperplasia, basal cell hyperplasia, degeneration, necrosis, and hyaline droplet accumulation. However, in general, nasal lesions were more prominent in the mice exposed to propargyl alcohol. Higher incidences and severities of nonneoplastic lesions were observed at lower exposure concentrations in mice than in rats. In addition, the incidences of nonneoplastic lesions were often higher in male rats and mice than in females. The reasons for the apparent sex and species differences are not known.

Nasal respiratory adenomas were observed in both sexes and both species. In the 2-year rat study, three of the males exposed to 64 ppm and one female exposed to 32 ppm developed nasal respiratory adenomas compared to none in the other exposed groups. In male rats, the trend test was statistically significant, and the incidence at 64 ppm exceeded the historical control ranges for inhalation studies and all study routes. In mice, exposure-related increases in the incidences of respiratory epithelial adenoma were noted in males and females. The incidences of nasal respiratory adenoma were significantly increased in male and female mice exposed to 32 ppm. Nasal respiratory neoplasms are extremely rare in control F344/N rats and B6C3F1 mice in NTP studies, and no nasal adenomas were reported in NTP historical control mice in the past 5 years. The increased incidences of nasal respiratory adenomas with an absence of malignant nasal tumors are considered to be some evidence of carcinogenicity in male rats and in male and female mice. Although there is some indirect evidence

that nasal adenomas may be able to undergo malignant progression (Brown *et al.*, 1991; IARC, 1992; Schwartz *et al.*, 1994; Morgan and Harkema, 1996), there was no evidence of progression in the current study.

A spectrum of nonneoplastic lesions occurred in the respiratory and olfactory epithelia of rats and mice at all exposure concentrations, and the severities of these lesions increased with increasing exposure concentration. The relationship of nonneoplastic proliferative lesions in the nasal cavity to nasal neoplasm formation is not clear. When hyperplasia is a regenerative or reparative response to degeneration and necrosis, it is not generally considered a preneoplastic lesion. However, it is not always clear histologically whether hyperplasia is a regenerative response or part of a morphological continuum to neoplasia (Boorman *et al.*, 1990). Incidences of degeneration and necrosis were generally low in the current 2-year studies. The NTP studied seven chemicals by the inhalation route that have produced neoplasms in the nasal cavity of rodents. All of these chemicals induced inflammatory and proliferative nasal lesions including inflammation, epithelial hyperplasia, and squamous metaplasia (Ward *et al*., 1993). Males were more sensitive than females to nasal neoplasm formation following exposure to three of these chemicals (allyl glycidyl ether, 1,2-epoxybutane, and furfuryl alcohol). However, of the 14 chemicals summarized by Ward *et al*. (1993) that were not carcinogenic in the nasal cavity, 12 produced similar inflammatory and proliferative nasal lesions. In the current study, most of the animals had nonneoplastic proliferative lesions in the nose, but only a few had nasal neoplasms. Therefore, it seems that the development of proliferative lesions alone is not sufficient for nasal neoplasm formation.

The nasal lesions in the current studies may have been related to enzymatic metabolism of propargyl alcohol by CYP2E1 in the nasal mucosal epithelium. Nasal epithelia are competent to metabolize foreign compounds. P450 enzymes have been localized in the nose, making the nasal cavity a ready target site for metabolite-induced lesions. Although the olfactory epithelium appears to be particularly vulnerable (Dahl and Lewis, 1993), the transitional epithelium of the anterior nasal cavity (Levels I and II) has also been reported to be rich in xenobiotic metabolizing enzymes (Dahl and Hadley, 1991), consistent with the abundant smooth endoplasmic reticulum present in these cells (Harkema and Morgan, 1996). In addition, the transitional epithelium predominantly lines the lateral walls and lateral aspects of the nasoturbinates and maxilloturbinates that border the lateral meatus, which receives a major portion of the nasal airflow in the rat (Morgan and Monticello, 1990; Harkema and Morgan, 1996). Finally, the water solubility of propargyl alcohol would be expected to enhance its absorption by the watery nasal secretions, favoring its uptake in the proximal nasal cavity where the transitional epithelium is located. For these reasons, nasal adenomas often arise, as in this study, on the lateral walls, nasoturbinates, and maxilloturbinates of the anterior nasal cavity (Brown *et al.*, 1991).

In addition to nasal lesions, propargyl alcohol exposure was associated with increased incidences of mononuclear cell leukemia in male rats. Although mononuclear cell leukemia is fairly common in control male rats (range of 38% to 66% for inhalation studies), there was an exposure concentration-related increase in the incidence of mononuclear cell leukemia in male rats, and the incidence was significantly increased in the 64 ppm group compared to the chamber controls. The incidence exceeded the NTP historical control ranges for inhalation studies and for all study routes. While the incidences increased with increasing exposure concentration in females, they did not reach statistical significance and were within the NTP historical control ranges. Ott *et al.* (1989a,b) reported that the exposure odds ratios for lymphoid and hematopoietic cancers were increased in humans exposed to the structurally related unsaturated alcohol, allyl alcohol, in a chemical manufacturing environment. However, the significance of this relationship was confounded by the exposure of employees to multiple chemicals.

The eye and Harderian gland lesions observed in male and/or female mice in the 2-year study were likely caused by propargyl alcohol exposure. Propargyl alcohol has previously been described as a skin and mucous membrane irritant. Effects in the eye were observed clinically in the 2-week and 2-year studies and histologically in the 2-year studies. In mice exposed to propargyl alcohol for 2 years, an increased incidence of cataract occurred in 32 ppm females, and increased incidences of corneal inflammation occurred in 32 ppm males and females. Significantly increased incidences of Harderian gland adenoma in male mice exposed to 8 or 32 ppm may have been related to propargyl alcohol exposure, and the incidences slightly exceeded the historical control range for inhalation studies. However, the incidences of Harderian gland carcinoma and Harderian gland adenoma or carcinoma (combined) were not significantly

increased in males. The incidences of Harderian gland neoplasms were not significantly increased in female mice. Harderian gland neoplasms have been observed previously after inhalation exposure of mice to a variety of NTP chemicals including 1,3-butadiene (NTP, 1993), chloroprene (NTP, 1998), ethylene oxide (NTP, 1987), and nitromethane (NTP, 1997). In these studies, the incidences were greater than those observed in the current study.

The individual animal data suggest that mice with Harderian gland neoplasms (of any type) appeared more likely to also have keratitis (corneal inflammation; 12 males and six females had both lesions). However, one male and seven female mice exposed to 32 ppm had keratitis without a Harderian gland neoplasm. These findings suggest propargyl alcohol had a direct irritant effect on the cornea, which may have been exacerbated by the presence of a Harderian gland neoplasm.

In summary, nasal lesions at all exposure concentrations were the primary exposure-related finding and included respiratory epithelium adenoma. Several proliferative and inflammatory nonneoplastic lesions occurred that involved the respiratory and olfactory epithelium at all exposure concentrations in males and females, and the lesions tended to be more severe in mice than in rats. An increased incidence of mononuclear cell leukemia occurred in male rats exposed to 64 ppm. The eyes of mice were also affected by exposure, most likely due to the irritant properties of propargyl alcohol. Incidences of Harderian gland neoplasms were increased in male mice exposed to 8 or 32 ppm.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity** of propargyl alcohol in male F344/N rats based on increased incidences of nasal respiratory epithelial adenoma and mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of propargyl alcohol in female F344/N rats exposed to 16, 32, or 64 ppm. There was *some evidence of carcinogenic activity* of propargyl alcohol in male and female B6C3F1 mice based on increased incidences of nasal respiratory epithelial adenoma. The increased incidences of Harderian gland adenoma in male B6C3F1 mice may have been related to exposure to propargyl alcohol.

Exposure to propargyl alcohol resulted in increased incidences of nonneoplastic nasal lesions in male and female rats and mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR INHALATION STUDY OF PROPARGYL ALCOHOL

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	16 ppm	32 ppm	64 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	20	19	26	30
Natural deaths	2	8	9	4
Survivors				
Died last week of study	1			
Terminal sacrifice	26	23	15	16
Missexed	1			
Animals examined microscopically	49	50	50	50
Alimentary System				
Intestine large, colon	(47)	(46)	(45)	(49)
Intestine large, rectum	(47)	(47)	(46)	(50)
Adenoma		1 (2%)		1 (2%)
Intestine small, jejunum	(46)	(45)	(45)	(48)
Adenoma		1 (2%)		
Liver	(49)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin				1 (2%)
Hepatocellular adenoma		1 (2%)		
Hepatocellular carcinoma	1 (2%)			
Mesentery	(11)	(14)	(7)	(3)
Oral mucosa	(0)	(1)	(1)	(0)
Squamous cell carcinoma		1 (100%)		
Squamous cell papilloma			1 (100%)	
Pancreas	(49)	(50)	(50)	(50)
Mixed tumor benign	2 (4%)			1 (2%)
Salivary glands	(49)	(50)	(50)	(50)
Stomach, forestomach	(49)	(50)	(50)	(50)
Stomach, glandular	(49)	(50)	(50)	(50)
Tongue	(1)	(0)	(2)	(1)
Squamous cell papilloma			1 (50%)	
Cardiovascular System				
Heart	(49)	(50)	(50)	(50)
Schwannoma benign	1 (2%)			1 (2%)
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Adenoma				2 (4%)
Adrenal medulla	(49)	(50)	(50)	(50)
Pheochromocytoma benign	6 (12%)	10 (20%)	3 (6%)	8 (16%)
Pheochromocytoma complex			1 (2%)	
Pheochromocytoma malignant	3 (6%)	2 (4%)		1 (2%)
Bilateral, pheochromocytoma benign	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Adenoma	4 (8%)	3 (6%)	6 (12%)	4 (8%)
Carcinoma	5 (10%)	4 (8%)	3 (6%)	4 (8%)
Parathyroid gland	(47)	(44)	(49)	(50)
Pituitary gland	(49)	(50)	(50)	(50)
Pars distalis, adenoma	36 (73%)	38 (76%)	34 (68%)	32 (64%)
Pars intermedia, adenoma	1 (2%)			

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Endocrine System (continued)				
Thyroid gland	(49)	(50)	(50)	(50)
Bilateral, C-cell, adenoma		1 (2%)		
C-cell, adenoma	4 (8%)	8 (16%)	7 (14%)	7 (14%)
C-cell, carcinoma	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Follicular cell, adenoma	1 (2%)	2 (4%)		
Follicular cell, carcinoma				2 (4%)
General Body System				
Peritoneum	(1)	(0)	(0)	(0)
Tissue, NOS	(1)	(0)	(0)	(0)
Thoracic, lipoma	1 (100%)			
Genital System				
Coagulating gland	(0)	(0)	(1)	(0)
Epididymis	(49)	(50)	(50)	(50)
Penis	(0)	(0)	(0)	(1)
Preputial gland	(49)	(49)	(50)	(49)
Adenoma	3 (6%)		1 (2%)	6 (12%)
Carcinoma				1 (2%)
Prostate	(49)	(50)	(50)	(50)
Seminal vesicle	(49)	(50)	(50)	(50)
Testes	(49)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	27 (55%)	21 (42%)	15 (30%)	31 (62%)
Interstitial cell, adenoma	11 (22%)	16 (32%)	18 (36%)	11 (22%)
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Lymph node	(5)	(3)	(7)	(8)
Lymph node, bronchial	(6)	(4)	(8)	(7)
Lymph node, mandibular	(0)	(1)	(1)	(0)
Lymph node, mediastinal	(24)	(24)	(24)	(26)
Lymph node, mesenteric	(49)	(50)	(50)	(49)
Spleen	(49)	(50)	(49)	(49)
Thymus	(47)	(43)	(46)	(45)
Thymoma benign	1 (2%)			
Integumentary System				
Mammary gland	(30)	(29)	(28)	(34)
Adenoma	2 (7%)		2 (7%)	
Fibroadenoma	1 (3%)		3 (11%)	1 (3%)
Skin	(49)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Keratoacanthoma	3 (6%)	5 (10%)	2 (4%)	4 (8%)
Keratoacanthoma, multiple	1 (2%)			
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma		1 (2%)		2 (4%)
Sebaceous gland, adenoma	1 (2%)			
Subcutaneous tissue, fibroma	3 (6%)	3 (6%)	3 (6%)	2 (4%)
Subcutaneous tissue, fibrosarcoma	1 (2%)			2 (4%)
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		1 (2%)
Subcutaneous tissue, lipoma	2 (4%)			
Subcutaneous tissue, osteosarcoma			1 (2%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)			

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Chordoma	1 (2%)			
Osteosarcoma	1 (2%)	1 (2%)		1 (2%)
Nervous System				
Brain	(49)	(50)	(50)	(50)
Ependymoma benign		1 (2%)		
Granular cell tumor benign				1 (2%)
Oligodendroglioma benign				1 (2%)
Respiratory System				
Larynx	(48)	(50)	(50)	(50)
Lung	(49)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)		2 (4%)
Alveolar/bronchiolar carcinoma		1 (2%)		
Chordoma, metastatic, bone	1 (2%)			
Fibrous histiocytoma, metastatic, skin				1 (2%)
Neural crest tumor, metastatic, skin	1 (2%)			
Osteosarcoma, metastatic, bone		1 (2%)		1 (2%)
Pheochromocytoma malignant, metastatic, adrenal medulla				1 (2%)
Alveolar epithelium, adenoma	1 (2%)			
Nose	(49)	(49)	(50)	(49)
Respiratory epithelium, adenoma				3 (6%)
Pleura	(0)	(1)	(0)	(0)
Mesothelium, osteosarcoma, metastatic, bone		1 (100%)		
Special Senses System				
Ear	(1)	(0)	(0)	(0)
Pinna, neural crest tumor	1 (100%)			
Eye	(49)	(50)	(50)	(50)
Zymbal's gland	(0)	(1)	(0)	(0)
Adenoma		1 (100%)		
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Urinary bladder	(49)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(49)	(50)	(50)	(50)
Histiocytic sarcoma		2 (4%)		2 (4%)
Leukemia mononuclear	21 (43%)	26 (52%)	23 (46%)	37 (74%)
Mesothelioma benign				1 (2%)
Mesothelioma malignant	1 (2%)			

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	50	49	49
Total primary neoplasms	154	160	128	177
Total animals with benign neoplasms	48	50	47	48
Total benign neoplasms	115	119	99	122
Total animals with malignant neoplasms	32	33	24	42
Total malignant neoplasms	38	41	29	54
Total animals with metastatic neoplasms	2	1		3
Total metastatic neoplasms	2	2		4
Total animals with uncertain neoplasms-benign or malignant	1			
Total uncertain neoplasms	1			

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

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	8/49 (16%)	12/50 (24%)	5/50 (10%)	9/50 (18%)
Adjusted rate[b]	18.8%	28.1%	12.7%	22.3%
Terminal rate[c]	4/27 (15%)	6/23 (26%)	2/15 (13%)	2/16 (13%)
First incidence (days)	589	485	680	544
Poly-3 test[d]	P=0.532N	P=0.224	P=0.324N	P=0.455
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	3/49 (6%)	2/50 (4%)	0/50 (0%)	1/50 (2%)
Adjusted rate	7.2%	4.9%	0.0%	2.6%
Terminal rate	2/27 (7%)	2/23 (9%)	0/15 (0%)	0/16 (0%)
First incidence (days)	720	729 (T)	—[e]	676
Poly-3 test	P=0.172N	P=0.511N	P=0.131N	P=0.337N
Adrenal Medulla: Benign, Malignant, or Complex Pheochromocytoma				
Overall rate	10/49 (20%)	13/50 (26%)	5/50 (10%)	9/50 (18%)
Adjusted rate	23.6%	30.4%	12.7%	22.3%
Terminal rate	6/27 (22%)	7/23 (30%)	2/15 (13%)	2/16 (13%)
First incidence (days)	589	485	680	544
Poly-3 test	P=0.324N	P=0.317	P=0.161N	P=0.548N
Mammary Gland: Fibroadenoma				
Overall rate	1/49 (2%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.4%	0.0%	7.7%	2.6%
Terminal rate	0/27 (0%)	0/23 (0%)	3/15 (20%)	1/16 (6%)
First incidence (days)	628	—	729 (T)	729 (T)
Poly-3 test	P=0.421	P=0.506N	P=0.277	P=0.738
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	3/49 (6%)	0/50 (0%)	5/50 (10%)	1/50 (2%)
Adjusted rate	7.1%	0.0%	12.8%	2.6%
Terminal rate	2/27 (7%)	0/23 (0%)	5/15 (33%)	1/16 (6%)
First incidence (days)	628	—	729 (T)	729 (T)
Poly-3 test	P=0.478N	P=0.124N	P=0.313	P=0.342N
Nose: Adenoma				
Overall rate	0/49 (0%)	0/49 (0%)	0/50 (0%)	3/49 (6%)
Adjusted rate	0.0%	0.0%	0.0%	7.9%
Terminal rate	0/27 (0%)	0/23 (0%)	0/15 (0%)	1/16 (6%)
First incidence (days)	—	—[f]	—	661
Poly-3 test	P=0.010	—	—	P=0.102
Pancreatic Islets: Adenoma				
Overall rate	4/49 (8%)	3/50 (6%)	6/50 (12%)	4/50 (8%)
Adjusted rate	9.6%	7.1%	15.2%	10.5%
Terminal rate	4/27 (15%)	1/23 (4%)	2/15 (13%)	3/16 (19%)
First incidence (days)	729 (T)	418	654	673
Poly-3 test	P=0.410	P=0.495N	P=0.331	P=0.595
Pancreatic Islets: Carcinoma				
Overall rate	5/49 (10%)	4/50 (8%)	3/50 (6%)	4/50 (8%)
Adjusted rate	11.6%	9.8%	7.7%	10.3%
Terminal rate	1/27 (4%)	3/23 (13%)	2/15 (13%)	2/16 (13%)
First incidence (days)	481	659	703	591
Poly-3 test	P=0.482N	P=0.530N	P=0.411N	P=0.565N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	9/49 (18%)	7/50 (14%)	9/50 (18%)	8/50 (16%)
Adjusted rate	20.9%	16.5%	22.8%	20.5%
Terminal rate	5/27 (19%)	4/23 (17%)	4/15 (27%)	5/16 (31%)
First incidence (days)	481	418	654	591
Poly-3 test	P=0.491	P=0.405N	P=0.523	P=0.591N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	36/49 (73%)	38/50 (76%)	34/50 (68%)	32/50 (64%)
Adjusted rate	76.8%	78.7%	76.9%	72.9%
Terminal rate	20/27 (74%)	18/23 (78%)	14/15 (93%)	14/16 (88%)
First incidence (days)	376	418	509	481
Poly-3 test	P=0.328N	P=0.510	P=0.601	P=0.421N
Preputial Gland: Adenoma				
Overall rate	3/49 (6%)	0/49 (0%)	1/50 (2%)	6/49 (12%)
Adjusted rate	7.1%	0.0%	2.6%	15.4%
Terminal rate	2/27 (7%)	0/23 (0%)	1/15 (7%)	3/16 (19%)
First incidence (days)	561	—	729 (T)	486
Poly-3 test	P=0.042	P=0.128N	P=0.335N	P=0.201
Preputial Gland: Adenoma or Carcinoma				
Overall rate	3/49 (6%)	0/49 (0%)	1/50 (2%)	7/49 (14%)
Adjusted rate	7.1%	0.0%	2.6%	17.9%
Terminal rate	2/27 (7%)	0/23 (0%)	1/15 (7%)	3/16 (19%)
First incidence (days)	561	—	729 (T)	486
Poly-3 test	P=0.016	P=0.128N	P=0.335N	P=0.124
Skin: Keratoacanthoma				
Overall rate	4/49 (8%)	5/50 (10%)	2/50 (4%)	4/50 (8%)
Adjusted rate	9.5%	12.2%	5.1%	10.5%
Terminal rate	2/27 (7%)	3/23 (13%)	2/15 (13%)	3/16 (19%)
First incidence (days)	624	684	729 (T)	661
Poly-3 test	P=0.535N	P=0.481	P=0.374N	P=0.589
Skin: Basal Cell Adenoma				
Overall rate	1/49 (2%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.4%	7.3%	2.5%	2.6%
Terminal rate	1/27 (4%)	2/23 (9%)	0/15 (0%)	1/16 (6%)
First incidence (days)	729 (T)	701	565	729 (T)
Poly-3 test	P=0.483N	P=0.297	P=0.749	P=0.740
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	4/49 (8%)	6/50 (12%)	2/50 (4%)	6/50 (12%)
Adjusted rate	9.5%	14.6%	5.1%	15.6%
Terminal rate	2/27 (7%)	3/23 (13%)	2/15 (13%)	4/16 (25%)
First incidence (days)	624	669	729 (T)	646
Poly-3 test	P=0.342	P=0.353	P=0.374N	P=0.312
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	4/49 (8%)	6/50 (12%)	2/50 (4%)	7/50 (14%)
Adjusted rate	9.5%	14.6%	5.1%	18.2%
Terminal rate	2/27 (7%)	3/23 (13%)	2/15 (13%)	5/16 (31%)
First incidence (days)	624	669	729 (T)	646
Poly-3 test	P=0.219	P=0.353	P=0.374N	P=0.208

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, or Squamous Cell Carcinoma				
Overall rate	5/49 (10%)	9/50 (18%)	3/50 (6%)	8/50 (16%)
Adjusted rate	11.8%	21.8%	7.6%	20.8%
Terminal rate	3/27 (11%)	5/23 (22%)	2/15 (13%)	6/16 (38%)
First incidence (days)	624	669	565	646
Poly-3 test	P=0.300	P=0.176	P=0.393N	P=0.215
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	3/49 (6%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	7.1%	7.2%	7.6%	5.3%
Terminal rate	2/27 (7%)	1/23 (4%)	0/15 (0%)	2/16 (13%)
First incidence (days)	565	614	633	729 (T)
Poly-3 test	P=0.450N	P=0.655	P=0.631	P=0.549N
Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Fibrosarcoma				
Overall rate	1/49 (2%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.4%	2.5%	0.0%	7.8%
Terminal rate	0/27 (0%)	1/23 (4%)	0/15 (0%)	0/16 (0%)
First incidence (days)	561	729 (T)	—	611
Poly-3 test	P=0.146	P=0.753	P=0.516N	P=0.273
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, or Fibrosarcoma				
Overall rate	4/49 (8%)	4/50 (8%)	3/50 (6%)	5/50 (10%)
Adjusted rate	9.3%	9.6%	7.6%	12.9%
Terminal rate	2/27 (7%)	2/23 (9%)	0/15 (0%)	2/16 (13%)
First incidence (days)	561	614	633	611
Poly-3 test	P=0.371	P=0.627	P=0.544N	P=0.436
Testes: Adenoma				
Overall rate	38/49 (78%)	37/50 (74%)	33/50 (66%)	42/50 (84%)
Adjusted rate	83.7%	81.7%	74.0%	91.2%
Terminal rate	25/27 (93%)	19/23 (83%)	11/15 (73%)	16/16 (100%)
First incidence (days)	503	504	558	486
Poly-3 test	P=0.171	P=0.513N	P=0.171N	P=0.189
Thyroid Gland (C-Cell): Adenoma				
Overall rate	4/49 (8%)	9/50 (18%)	7/50 (14%)	7/50 (14%)
Adjusted rate	9.5%	22.0%	17.6%	17.7%
Terminal rate	1/27 (4%)	8/23 (35%)	3/15 (20%)	2/16 (13%)
First incidence (days)	673	684	626	565
Poly-3 test	P=0.285	P=0.101	P=0.225	P=0.222
Thyroid Gland (C-Cell): Carcinoma				
Overall rate	3/49 (6%)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rate	7.2%	7.3%	2.5%	5.3%
Terminal rate	3/27 (11%)	2/23 (9%)	0/15 (0%)	1/16 (6%)
First incidence (days)	729 (T)	701	607	711
Poly-3 test	P=0.372N	P=0.653	P=0.326N	P=0.542N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	7/49 (14%)	12/50 (24%)	8/50 (16%)	9/50 (18%)
Adjusted rate	16.6%	29.2%	19.9%	22.8%
Terminal rate	4/27 (15%)	10/23 (44%)	3/15 (20%)	3/16 (19%)
First incidence (days)	673	684	607	565
Poly-3 test	P=0.427	P=0.131	P=0.460	P=0.337

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
All Organs: Mononuclear Cell Leukemia				
Overall rate	21/49 (43%)	26/50 (52%)	23/50 (46%)	37/50 (74%)
Adjusted rate	48.2%	57.6%	52.5%	80.1%
Terminal rate	12/27 (44%)	11/23 (48%)	5/13 (33%)	11/16 (69%)
First incidence (days)	589	418	523	481
Poly-3 test	P<0.001	P=0.246	P=0.424	P<0.001
All Organs: Benign Neoplasms				
Overall rate	48/49 (98%)	50/50 (100%)	47/50 (94%)	48/50 (96%)
Adjusted rate	98.9%	100.0%	97.9%	98.8%
Terminal rate	27/27 (100%)	23/23 (100%)	15/15 (100%)	16/16 (100%)
First incidence (days)	376	418	509	481
Poly-3 test	P=0.603N	P=0.810	P=0.729N	P=0.903N
All Organs: Malignant Neoplasms				
Overall rate	32/49 (65%)	33/50 (66%)	24/50 (48%)	42/50 (84%)
Adjusted rate	69.7%	71.7%	54.8%	89.2%
Terminal rate	17/27 (63%)	15/23 (65%)	6/15 (40%)	13/16 (81%)
First incidence (days)	481	418	523	481
Poly-3 test	P=0.018	P=0.511	P=0.097N	P=0.013
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/49 (100%)	50/50 (100%)	49/50 (98%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	99.9%	99.9%
Terminal rate	27/27 (100%)	23/23 (100%)	15/15 (100%)	16/16 (100%)
First incidence (days)	376	418	509	481
Poly-3 test	P=1.000N	—	P=1.000N	P=1.000N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal medulla, nose, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE A3a
Historical Incidence of Adenoma of the Nose in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Inhalation Studies	
Cumene	0/50
Decalin	0/49
Divinylbenzene	0/50
Methyl isobutyl ketone	0/50
α-Methylstyrene	0/50
Propargyl alcohol	0/49
Propylene glycol mono-*t*-butyl ether	0/50
Stoddard solvent (Type IIC)	1/50
Vanadium pentoxide	0/49
Overall Historical Incidence: Inhalation Studies	
Total (%)	1/447 (0.2%)
Mean ± standard deviation	0.2% ± 0.7%
Range	0%-2%
Overall Historical Incidence: All Routes	
Total (%)	2/1,439 (0.1%)
Mean ± standard deviation	0.1% ± 0.5%
Range	0%-2%

[a] Data as of March 5, 2007

TABLE A3b
Historical Incidence of Mononuclear Cell Leukemia in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Inhalation Studies	
Cumene	26/50
Decalin	19/50
Divinylbenzene	22/50
Methyl isobutyl ketone	25/50
α-Methylstyrene	26/50
Propargyl alcohol	21/49
Propylene glycol mono-*t*-butyl ether	33/50
Stoddard solvent (Type IIC)	25/50
Vanadium pentoxide	22/50
Overall Historical Incidence: Inhalation Studies	
Total (%)	219/449 (48.8%)
Mean ± standard deviation	48.8% ± 8.0%
Range	38%-66%
Overall Historical Incidence: All Routes	
Total (%)	559/1,449 (38.6%)
Mean ± standard deviation	38.5% ± 12.0%
Range	18%-66%

[a] Data as of March 5, 2007; includes data for lymphocytic, monocytic, mononuclear, or undifferentiated leukemia

TABLE A3c
Historical Incidence of Preputial Gland Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls	
	Adenoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies		
Cumene	0/50	0/50
Decalin	0/50	0/50
Divinylbenzene	0/48	0/48
Methyl isobutyl ketone	0/50	1/50
α-Methylstyrene	0/50	2/50
Propargyl alcohol	3/49	3/49
Propylene glycol mono-*t*-butyl ether	1/50	2/50
Stoddard solvent (Type IIC)	0/50	0/50
Vanadium pentoxide	0/50	0/50
Overall Historical Incidence: Inhalation Studies		
Total (%)	4/447 (0.9%)	8/447 (1.8%)
Mean ± standard deviation	0.9% ± 2.1%	1.8% ± 2.4%
Range	0%-6%	0%-6%
Overall Historical Incidence: All Routes		
Total (%)	49/1,442 (3.4%)	75/1,442 (5.2%)
Mean ± standard deviation	3.3% ± 2.8%	5.0% ± 3.3%
Range	0%-10%	0%-12%

[a] Data as of March 5, 2007

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	16 ppm	32 ppm	64 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	20	19	26	30
Natural deaths	2	8	9	4
Survivors				
Died last week of study	1			
Terminal sacrifice	26	23	15	16
Missexed	1			
Animals examined microscopically	49	50	50	50
Alimentary System				
Intestine large, colon	(47)	(46)	(45)	(49)
Artery, inflammation, chronic active	1 (2%)			
Intestine large, rectum	(47)	(47)	(46)	(50)
Edema	1 (2%)			
Inflammation, suppurative	1 (2%)			
Intestine small, jejunum	(46)	(45)	(45)	(48)
Liver	(49)	(50)	(50)	(50)
Angiectasis	1 (2%)	3 (6%)		
Basophilic focus	7 (14%)	18 (36%)	15 (30%)	4 (8%)
Clear cell focus	10 (20%)	13 (26%)	9 (18%)	4 (8%)
Degeneration, cystic	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Eosinophilic focus	2 (4%)	4 (8%)	2 (4%)	5 (10%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)	1 (2%)	
Hemorrhage			1 (2%)	
Hepatodiaphragmatic nodule		5 (10%)	2 (4%)	1 (2%)
Inflammation, granulomatous			1 (2%)	
Inflammation, chronic active	1 (2%)		1 (2%)	
Mixed cell focus	2 (4%)	4 (8%)	5 (10%)	3 (6%)
Necrosis	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Vacuolization cytoplasmic	7 (14%)	5 (10%)	3 (6%)	3 (6%)
Centrilobular, degeneration	1 (2%)			1 (2%)
Hepatocyte, regeneration				2 (4%)
Mesentery	(11)	(14)	(7)	(3)
Necrosis	10 (91%)	14 (100%)	7 (100%)	2 (67%)
Thrombosis				1 (33%)
Artery, inflammation, chronic active	1 (9%)			
Oral mucosa	(0)	(1)	(1)	(0)
Pancreas	(49)	(50)	(50)	(50)
Acinus, atrophy	24 (49%)	21 (42%)	22 (44%)	26 (52%)
Acinus, hyperplasia	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Duct, necrosis			1 (2%)	
Salivary glands	(49)	(50)	(50)	(50)
Stomach, forestomach	(49)	(50)	(50)	(50)
Edema	1 (2%)			
Erosion		1 (2%)		1 (2%)
Hyperplasia, squamous		3 (6%)	3 (6%)	
Inflammation, chronic			1 (2%)	
Ulcer	4 (8%)	3 (6%)	3 (6%)	2 (4%)
Stomach, glandular	(49)	(50)	(50)	(50)
Erosion	4 (8%)	2 (4%)	5 (10%)	3 (6%)
Ulcer	5 (10%)		1 (2%)	4 (8%)
Tongue	(1)	(0)	(2)	(1)

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Cardiovascular System				
Heart	(49)	(50)	(50)	(50)
Cardiomyopathy	44 (90%)	44 (88%)	44 (88%)	43 (86%)
Atrium, thrombosis	3 (6%)	6 (12%)	8 (16%)	5 (10%)
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Degeneration, cystic	1 (2%)			
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia	19 (39%)	23 (46%)	27 (54%)	28 (56%)
Hypertrophy	1 (2%)			
Thrombosis			1 (2%)	
Vacuolization cytoplasmic	11 (22%)	8 (16%)	9 (18%)	7 (14%)
Subcapsular, hyperplasia	1 (2%)			
Adrenal medulla	(49)	(50)	(50)	(50)
Hyperplasia	15 (31%)	19 (38%)	21 (42%)	18 (36%)
Infiltration cellular, lymphocyte	1 (2%)			
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia	1 (2%)	5 (10%)	1 (2%)	1 (2%)
Parathyroid gland	(47)	(44)	(49)	(50)
Hyperplasia		2 (5%)		
Pituitary gland	(49)	(50)	(50)	(50)
Hemorrhage		1 (2%)	3 (6%)	
Pars distalis, hyperplasia	8 (16%)	6 (12%)	6 (12%)	13 (26%)
Pars intermedia, cyst				1 (2%)
Thyroid gland	(49)	(50)	(50)	(50)
Cyst		1 (2%)		
C-cell, hyperplasia	8 (16%)	6 (12%)	15 (30%)	12 (24%)
Follicular cell, hyperplasia		1 (2%)		1 (2%)
General Body System				
Peritoneum	(1)	(0)	(0)	(0)
Tissue, NOS	(1)	(0)	(0)	(0)
Genital System				
Coagulating gland	(0)	(0)	(1)	(0)
Inflammation, suppurative			1 (100%)	
Epididymis	(49)	(50)	(50)	(50)
Penis	(0)	(0)	(0)	(1)
Inflammation, suppurative				1 (100%)
Preputial gland	(49)	(49)	(50)	(49)
Cyst			1 (2%)	
Hyperplasia				2 (4%)
Inflammation, chronic active	18 (37%)	12 (24%)	11 (22%)	12 (24%)
Necrosis				1 (2%)
Prostate	(49)	(50)	(50)	(50)
Hyperplasia	9 (18%)	2 (4%)	10 (20%)	8 (16%)
Inflammation, suppurative	31 (63%)	41 (82%)	36 (72%)	34 (68%)
Inflammation, chronic active	4 (8%)		1 (2%)	2 (4%)
Seminal vesicle	(49)	(50)	(50)	(50)
Hyperplasia	1 (2%)			
Testes	(49)	(50)	(50)	(50)
Germinal epithelium, atrophy	6 (12%)	3 (6%)	3 (6%)	4 (8%)
Interstitial cell, hyperplasia	4 (8%)	4 (8%)	7 (14%)	5 (10%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Myelofibrosis		1 (2%)		
Erythroid cell, hyperplasia		1 (2%)	2 (4%)	1 (2%)
Lymph node	(5)	(3)	(7)	(8)
Deep cervical, hemorrhage	1 (20%)	1 (33%)	1 (14%)	
Deep cervical, hyperplasia, lymphoid	1 (20%)			
Pancreatic, angiectasis	2 (40%)			2 (25%)
Pancreatic, infiltration cellular, histiocyte			1 (14%)	
Lymph node, bronchial	(6)	(4)	(8)	(7)
Angiectasis	1 (17%)		2 (25%)	
Hemorrhage			1 (13%)	
Hyperplasia, lymphoid	1 (17%)	2 (50%)	1 (13%)	
Lymph node, mandibular	(0)	(1)	(1)	(0)
Hyperplasia, lymphoid		1 (100%)		
Lymph node, mediastinal	(24)	(24)	(24)	(26)
Angiectasis		1 (4%)		
Hemorrhage		1 (4%)		
Hyperplasia, lymphoid	3 (13%)	1 (4%)	3 (13%)	2 (8%)
Infiltration, cellular, histiocyte	1 (4%)			
Inflammation, chronic active		1 (4%)		
Lymph node, mesenteric	(49)	(50)	(50)	(49)
Angiectasis	1 (2%)			
Ectasia	1 (2%)			
Hemorrhage	1 (2%)			
Hyperplasia, lymphoid	1 (2%)		2 (4%)	
Infiltration cellular, histiocyte	9 (18%)	5 (10%)	5 (10%)	3 (6%)
Spleen	(49)	(50)	(49)	(49)
Angiectasis			1 (2%)	
Hematopoietic cell proliferation	8 (16%)	10 (20%)	5 (10%)	8 (16%)
Hemorrhage			1 (2%)	
Hemorrhage, chronic	1 (2%)	3 (6%)	2 (4%)	
Hyperplasia, lymphoid, focal	1 (2%)			
Hyperplasia, lymphoid			2 (4%)	
Infarct			1 (2%)	
Thymus	(47)	(43)	(46)	(45)
Cyst				1 (2%)
Integumentary System				
Mammary gland	(30)	(29)	(28)	(34)
Galactocele		1 (3%)	1 (4%)	3 (9%)
Inflammation, suppurative	1 (3%)			
Skin	(49)	(50)	(50)	(50)
Cyst epithelial inclusion	2 (4%)		3 (6%)	
Ulcer	5 (10%)	1 (2%)	2 (4%)	1 (2%)
Subcutaneous tissue, fibrosis	1 (2%)			
Subcutaneous tissue, inflammation	1 (2%)			
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Hyperostosis		1 (2%)	1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Nervous System				
Brain	(49)	(50)	(50)	(50)
Compression	10 (20%)	9 (18%)	18 (36%)	9 (18%)
Hemorrhage		1 (2%)	3 (6%)	2 (4%)
Necrosis	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Thrombosis				1 (2%)
Respiratory System				
Larynx	(48)	(50)	(50)	(50)
Foreign body	2 (4%)	3 (6%)	1 (2%)	
Inflammation, chronic active	3 (6%)			
Metaplasia, squamous	1 (2%)		1 (2%)	
Lung	(49)	(50)	(50)	(50)
Foreign body	1 (2%)			
Hemorrhage		1 (2%)	3 (6%)	2 (4%)
Inflammation		1 (2%)		2 (4%)
Thrombosis			1 (2%)	1 (2%)
Alveolar epithelium, hyperplasia	8 (16%)	12 (24%)	11 (22%)	7 (14%)
Alveolar epithelium, metaplasia, squamous	2 (4%)	1 (2%)		1 (2%)
Alveolus, infiltration cellular, histiocyte	4 (8%)	1 (2%)		1 (2%)
Nose	(49)	(49)	(50)	(49)
Foreign body	7 (14%)	8 (16%)	6 (12%)	8 (16%)
Hemorrhage				1 (2%)
Inflammation, suppurative	1 (2%)	1 (2%)		
Inflammation, chronic active	9 (18%)	12 (24%)	22 (44%)	28 (57%)
Glands, olfactory epithelium, hyperplasia				4 (8%)
Glands, respiratory epithelium, hyperplasia	3 (6%)	14 (29%)	39 (78%)	45 (92%)
Olfactory epithelium, accumulation, hyaline droplet		5 (10%)	4 (8%)	7 (14%)
Olfactory epithelium, atrophy	1 (2%)	21 (43%)	26 (52%)	26 (53%)
Olfactory epithelium, degeneration			1 (2%)	7 (14%)
Olfactory epithelium, hyperplasia		1 (2%)	3 (6%)	5 (10%)
Olfactory epithelium, hyperplasia, basal cell		19 (39%)	42 (84%)	42 (86%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)	10 (20%)	18 (36%)	29 (59%)
Olfactory epithelium, necrosis			2 (4%)	6 (12%)
Respiratory epithelium, hyperplasia	5 (10%)	21 (43%)	44 (88%)	42 (86%)
Respiratory epithelium, metaplasia, squamous	2 (4%)	2 (4%)	2 (4%)	4 (8%)
Pleura	(0)	(1)	(0)	(0)
Special Senses System				
Ear	(1)	(0)	(0)	(0)
Eye	(49)	(50)	(50)	(50)
Inflammation, chronic active			1 (2%)	
Cornea, mineralization	1 (2%)			
Lens, cataract	1 (2%)	3 (6%)	2 (4%)	
Zymbal's gland	(0)	(1)	(0)	(0)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Cyst	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Infarct	1 (2%)	1 (2%)	2 (4%)	
Inflammation, suppurative	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Nephropathy, chronic	42 (86%)	47 (94%)	48 (96%)	48 (96%)
Transitional epithelium, infarct			1 (2%)	
Urinary bladder	(49)	(50)	(50)	(50)
Edema				1 (2%)
Inflammation, chronic	1 (2%)		1 (2%)	
Transitional epithelium, hyperplasia	1 (2%)			

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF PROPARGYL ALCOHOL

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	16 ppm	32 ppm	64 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	18	16	19	19
Natural deaths	3	3	4	5
Survivors				
Died last week of study		1		
Terminal sacrifice	29	30	27	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Fibrosarcoma	1 (2%)			
Intestine large, cecum	(48)	(48)	(47)	(46)
Intestine large, colon	(48)	(48)	(47)	(47)
Intestine small, duodenum	(49)	(50)	(48)	(47)
Intestine small, ileum	(48)	(48)	(46)	(46)
Intestine small, jejunum	(48)	(48)	(47)	(46)
Liver	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin			1 (2%)	
Hepatocellular adenoma				1 (2%)
Mesentery	(13)	(12)	(10)	(9)
Oral mucosa	(1)	(0)	(2)	(1)
Squamous cell carcinoma				1 (100%)
Squamous cell papilloma			1 (50%)	
Pancreas	(50)	(50)	(50)	(50)
Acinus, adenoma			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(49)	(50)	(50)	(48)
Tongue	(0)	(0)	(2)	(1)
Squamous cell carcinoma			1 (50%)	
Squamous cell papilloma				1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma benign				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	2 (4%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)		2 (4%)	
Parathyroid gland	(49)	(47)	(50)	(47)
Adenoma		1 (2%)		
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	30 (60%)	32 (64%)	31 (62%)	29 (58%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma	1 (2%)			1 (2%)
C-cell, adenoma	2 (4%)	9 (18%)	2 (4%)	5 (10%)
C-cell, carcinoma	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Follicular cell, adenoma		2 (4%)		
Follicular cell, carcinoma		1 (2%)		1 (2%)
General Body System				
Tissue, NOS	(1)	(0)	(1)	(0)
Genital System				
Clitoral gland	(50)	(49)	(49)	(47)
Adenoma	4 (8%)	3 (6%)	5 (10%)	3 (6%)
Adenoma, multiple			1 (2%)	1 (2%)
Papilloma		5 (10%)	1 (2%)	1 (2%)
Schwannoma malignant, metastatic, skin		1 (2%)		
Ovary	(50)	(50)	(50)	(50)
Uterus	(50)	(50)	(50)	(50)
Carcinoma				2 (4%)
Hemangiosarcoma	1 (2%)			
Polyp stromal	12 (24%)	6 (12%)	9 (18%)	8 (16%)
Sarcoma stromal		2 (4%)	1 (2%)	1 (2%)
Cervix, granular cell tumor benign		1 (2%)		
Cervix, polyp stromal		2 (4%)		
Endometrium, adenoma			1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(4)	(0)	(3)	(3)
Lymph node, bronchial	(4)	(2)	(4)	(10)
Lymph node, mediastinal	(29)	(27)	(30)	(25)
Lymph node, mesenteric	(49)	(50)	(49)	(50)
Spleen	(50)	(50)	(50)	(49)
Hemangiosarcoma	1 (2%)			1 (2%)
Thymus	(46)	(45)	(47)	(48)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Carcinoma	7 (14%)	2 (4%)	4 (8%)	3 (6%)
Fibroadenoma	16 (32%)	15 (30%)	14 (28%)	15 (30%)
Fibroadenoma, multiple	10 (20%)	7 (14%)	10 (20%)	11 (22%)
Skin	(50)	(50)	(50)	(50)
Keratoacanthoma		1 (2%)		1 (2%)
Squamous cell papilloma				1 (2%)
Trichoepithelioma	1 (2%)			
Subcutaneous tissue, fibroma	1 (2%)		1 (2%)	2 (4%)
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	
Subcutaneous tissue, osteosarcoma				1 (2%)
Subcutaneous tissue, schwannoma malignant		1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(2)	(0)	(1)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant			1 (2%)	1 (2%)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)		1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)			
Carcinoma, metastatic, mammary gland				1 (2%)
Carcinoma, metastatic, Zymbal's gland			1 (2%)	
Fibrous histiocytoma, metastatic, skin			1 (2%)	
Osteosarcoma, metastatic, skin				1 (2%)
Nose	(49)	(49)	(50)	(50)
Glands, carcinoma		1 (2%)		
Respiratory epithelium, adenoma			1 (2%)	
Special Senses System				
Eye	(49)	(50)	(50)	(50)
Zymbal's gland	(0)	(0)	(2)	(1)
Carcinoma			2 (100%)	1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Renal tubule, adenoma		1 (2%)		
Urinary bladder	(49)	(50)	(50)	(49)
Systemic Lesions[b]				
Multiple organs	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Leukemia mononuclear	20 (40%)	13 (26%)	23 (46%)	26 (52%)
Mesothelioma benign	1 (2%)			
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	47	50	47
Total primary neoplasms	117	110	119	125
Total animals with benign neoplasms	42	45	43	41
Total benign neoplasms	82	89	84	85
Total animals with malignant neoplasms	26	20	29	33
Total malignant neoplasms	34	21	35	40
Total animals with metastatic neoplasms		1	2	2
Total metastatic neoplasms		1	3	2

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

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Clitoral Gland: Papilloma				
Overall rate[a]	0/50 (0%)	5/49 (10%)	1/49 (2%)	1/47 (2%)
Adjusted rate[b]	0.0%	11.5%	2.4%	2.5%
Terminal rate[c]	0/29 (0%)	4/30 (13%)	0/27 (0%)	1/24 (4%)
First incidence (days)	—[e]	677	708	730 (T)
Poly-3 test[d]	P=0.485N	P=0.034	P=0.501	P=0.489
Clitoral Gland: Adenoma				
Overall rate	4/50 (8%)	3/49 (6%)	6/49 (12%)	4/47 (9%)
Adjusted rate	9.5%	6.9%	14.3%	10.1%
Terminal rate	2/29 (7%)	1/30 (3%)	5/27 (19%)	3/24 (13%)
First incidence (days)	673	695	676	674
Poly-3 test	P=0.435	P=0.481N	P=0.370	P=0.613
Mammary Gland: Fibroadenoma				
Overall rate	26/50 (52%)	22/50 (44%)	24/50 (48%)	26/50 (52%)
Adjusted rate	57.2%	47.1%	53.0%	58.2%
Terminal rate	16/29 (55%)	14/31 (45%)	14/27 (52%)	15/26 (58%)
First incidence (days)	393	516	598	618
Poly-3 test	P=0.380	P=0.218N	P=0.421N	P=0.550
Mammary Gland: Adenoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
Adjusted rate	7.1%	2.3%	2.3%	2.4%
Terminal rate	0/29 (0%)	1/31 (3%)	0/27 (0%)	1/26 (4%)
First incidence (days)	652	730 (T)	712	730 (T)
Poly-3 test	P=0.248N	P=0.290N	P=0.301N	P=0.306N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	28/50 (56%)	22/50 (44%)	25/50 (50%)	26/50 (52%)
Adjusted rate	60.9%	47.1%	55.1%	58.2%
Terminal rate	16/29 (55%)	14/31 (45%)	14/27 (52%)	15/26 (58%)
First incidence (days)	393	516	598	618
Poly-3 test	P=0.490	P=0.125N	P=0.361N	P=0.480N
Mammary Gland: Carcinoma				
Overall rate	7/50 (14%)	2/50 (4%)	4/50 (8%)	3/50 (6%)
Adjusted rate	16.7%	4.5%	9.2%	6.9%
Terminal rate	5/29 (17%)	2/31 (7%)	1/27 (4%)	0/26 (0%)
First incidence (days)	680	730 (T)	598	516
Poly-3 test	P=0.186N	P=0.066N	P=0.239N	P=0.143N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	10/50 (20%)	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted rate	23.4%	6.8%	9.2%	9.2%
Terminal rate	5/29 (17%)	3/31 (10%)	1/27 (4%)	1/26 (4%)
First incidence (days)	652	730 (T)	598	516
Poly-3 test	P=0.083N	P=0.028N	P=0.065N	P=0.066N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	31/50 (62%)	23/50 (46%)	26/50 (52%)	27/50 (54%)
Adjusted rate	67.2%	49.2%	57.1%	59.6%
Terminal rate	18/29 (62%)	15/31 (48%)	14/27 (52%)	15/26 (58%)
First incidence (days)	393	516	598	516
Poly-3 test	P=0.424N	P=0.056N	P=0.212N	P=0.290N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	30/50 (60%)	32/50 (64%)	31/50 (62%)	29/50 (58%)
Adjusted rate	65.3%	67.7%	66.5%	63.1%
Terminal rate	18/29 (62%)	21/31 (68%)	18/27 (67%)	16/26 (62%)
First incidence (days)	513	516	509	617
Poly-3 test	P=0.416N	P=0.490	P=0.540	P=0.499N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	3/50 (6%)	9/50 (18%)	2/50 (4%)	6/50 (12%)
Adjusted rate	7.2%	20.1%	4.7%	14.0%
Terminal rate	3/29 (10%)	7/31 (23%)	1/27 (4%)	3/26 (12%)
First incidence (days)	730 (T)	627	696	681
Poly-3 test	P=0.453	P=0.075	P=0.486N	P=0.254
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	5/50 (10%)	10/50 (20%)	4/50 (8%)	6/50 (12%)
Adjusted rate	11.9%	22.3%	9.3%	14.0%
Terminal rate	4/29 (14%)	7/31 (23%)	1/27 (4%)	3/26 (12%)
First incidence (days)	633	627	682	681
Poly-3 test	P=0.447N	P=0.158	P=0.483N	P=0.513
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	0.0%	6.8%	0.0%	2.3%
Terminal rate	0/29 (0%)	3/31 (10%)	0/27 (0%)	0/26 (0%)
First incidence (days)	—	730 (T)	—[f]	522
Poly-3 test	P=0.619N	P=0.129	—	P=0.506
Uterus: Stromal Polyp				
Overall rate	12/50 (24%)	8/50 (16%)	9/50 (18%)	8/50 (16%)
Adjusted rate	28.0%	17.4%	20.4%	18.5%
Terminal rate	9/29 (31%)	3/31 (10%)	4/27 (15%)	4/26 (15%)
First incidence (days)	581	575	509	620
Poly-3 test	P=0.248N	P=0.174N	P=0.281N	P=0.213N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	12/50 (24%)	10/50 (20%)	10/50 (20%)	9/50 (18%)
Adjusted rate	28.0%	21.5%	22.3%	20.8%
Terminal rate	9/29 (31%)	3/31 (10%)	4/27 (15%)	5/26 (19%)
First incidence (days)	581	556	430	620
Poly-3 test	P=0.299N	P=0.319N	P=0.355N	P=0.297N
All Organs: Mononuclear Cell Leukemia				
Overall rate	20/50 (40%)	13/50 (26%)	23/50 (46%)	26/50 (52%)
Adjusted rate	45.0%	28.4%	49.1%	56.0%
Terminal rate	11/29 (38%)	8/31 (26%)	11/27 (41%)	13/26 (50%)
First incidence (days)	513	516	439	502
Poly-3 test	P=0.040	P=0.074N	P=0.427	P=0.195
All Organs: Benign Neoplasms				
Overall rate	42/50 (84%)	45/50 (90%)	43/50 (86%)	41/50 (82%)
Adjusted rate	87.0%	92.1%	90.4%	86.0%
Terminal rate	25/29 (86%)	28/31 (90%)	24/27 (89%)	22/26 (85%)
First incidence (days)	393	516	509	522
Poly-3 test	P=0.407N	P=0.306	P=0.416	P=0.568N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	20/50 (40%)	29/50 (58%)	33/50 (66%)
Adjusted rate	57.6%	42.8%	59.9%	68.8%
Terminal rate	15/29 (52%)	11/31 (36%)	12/27 (44%)	15/26 (58%)
First incidence (days)	513	516	430	502
Poly-3 test	P=0.043	P=0.108N	P=0.494	P=0.178
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/50 (94%)	47/50 (94%)	50/50 (100%)	47/50 (94%)
Adjusted rate	96.6%	95.1%	100.0%	94.0%
Terminal rate	28/29 (97%)	29/31 (94%)	27/27 (100%)	23/26 (89%)
First incidence (days)	393	516	430	502
Poly-3 test	P=0.405N	P=0.557N	P=0.267	P=0.449N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for clitoral gland, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE B3a
Historical Incidence of Mononuclear Cell Leukemia in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Inhalation Studies	
Cumene	12/50
Decalin	11/50
Divinylbenzene	10/50
Methyl isobutyl ketone	14/50
α-Methylstyrene	18/50
Propargyl alcohol	20/50
Propylene glycol mono-*t*-butyl ether	24/50
Stoddard solvent (Type IIC)	26/50
Vanadium pentoxide	21/50
Overall Historical Incidence: Inhalation Studies	
Total (%)	156/450 (34.7%)
Mean ± standard deviation	34.7% ± 11.7%
Range	20%-52%
Overall Historical Incidence: All Routes	
Total (%)	342/1,350 (25.3%)
Mean ± standard deviation	25.5% ± 10.9%
Range	10%-52%

[a] Data as of March 5, 2007; includes data for lymphocytic, monocytic, mononuclear, or undifferentiated leukemia

TABLE B3b
Historical Incidence of Squamous Cell Papilloma of the Clitoral Gland in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Inhalation Studies	
Cumene	0/50
Decalin	0/48
Divinylbenzene	0/50
Methyl isobutyl ketone	0/50
α-Methylstyrene	0/50
Propargyl alcohol	0/50
Propylene glycol mono-*t*-butyl ether	0/50
Stoddard solvent (Type IIC)	0/49
Vanadium pentoxide	0/50
Overall Historical Incidence: Inhalation Studies	
Total (%)	0/447 (0.0%)
Overall Historical Incidence: All Routes	
Total (%)	2/1,341 (0.2%)
Mean ± standard deviation	0.2% ± 0.5%
Range	0%-2%

[a] Data as of March 5, 2007

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	16 ppm	32 ppm	64 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	18	16	19	19
Natural deaths	3	3	4	5
Survivors				
Died last week of study		1		
Terminal sacrifice	29	30	27	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(48)	(48)	(47)	(46)
Ulcer		1 (2%)		
Serosa, inflammation	1 (2%)			
Intestine large, colon	(48)	(48)	(47)	(47)
Serosa, inflammation	1 (2%)			
Intestine small, duodenum	(49)	(50)	(48)	(47)
Serosa, inflammation	1 (2%)			
Intestine small, ileum	(48)	(48)	(46)	(46)
Intestine small, jejunum	(48)	(48)	(47)	(46)
Liver	(50)	(50)	(50)	(50)
Angiectasis		4 (8%)	1 (2%)	1 (2%)
Basophilic focus	37 (74%)	36 (72%)	40 (80%)	31 (62%)
Clear cell focus	6 (12%)	6 (12%)	7 (14%)	7 (14%)
Eosinophilic focus		2 (4%)	3 (6%)	3 (6%)
Fibrosis	1 (2%)			
Hepatodiaphragmatic nodule	3 (6%)	2 (4%)	5 (10%)	2 (4%)
Inflammation, granulomatous	1 (2%)			
Inflammation, chronic active	1 (2%)		2 (4%)	1 (2%)
Mixed cell focus	5 (10%)	4 (8%)	4 (8%)	12 (24%)
Necrosis		1 (2%)		2 (4%)
Vacuolization cytoplasmic	8 (16%)	3 (6%)	3 (6%)	4 (8%)
Centrilobular, necrosis	1 (2%)			1 (2%)
Oval cell, hyperplasia				1 (2%)
Mesentery	(13)	(12)	(10)	(9)
Necrosis	13 (100%)	12 (100%)	10 (100%)	9 (100%)
Oral mucosa	(1)	(0)	(2)	(1)
Foreign body	1 (100%)		1 (50%)	
Ulcer			1 (50%)	
Pancreas	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Acinus, atrophy	6 (12%)	9 (18%)	11 (22%)	14 (28%)
Acinus, inflammation, chronic				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Necrosis				1 (2%)
Ulcer	3 (6%)	5 (10%)	1 (2%)	2 (4%)
Epithelium, hyperplasia	1 (2%)	1 (2%)		1 (2%)
Serosa, inflammation	1 (2%)			

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Alimentary System (continued)				
Stomach, glandular	(49)	(50)	(50)	(48)
Erosion	3 (6%)	1 (2%)	1 (2%)	3 (6%)
Ulcer	1 (2%)		1 (2%)	1 (2%)
Serosa, inflammation	1 (2%)			
Tongue	(0)	(0)	(2)	(1)
Inflammation, granulomatous, chronic active				1 (100%)
Epithelium, hyperplasia			1 (50%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	43 (86%)	45 (90%)	42 (84%)	42 (84%)
Atrium, thrombosis	2 (4%)		2 (4%)	4 (8%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	3 (6%)			
Degeneration, cystic			1 (2%)	2 (4%)
Hemorrhage				1 (2%)
Hyperplasia	28 (56%)	23 (46%)	22 (44%)	21 (42%)
Necrosis	1 (2%)	1 (2%)		
Vacuolization cytoplasmic	3 (6%)	3 (6%)	1 (2%)	4 (8%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)	7 (14%)	3 (6%)	2 (4%)
Necrosis	1 (2%)			
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	4 (8%)	4 (8%)	
Parathyroid gland	(49)	(47)	(50)	(47)
Pituitary gland	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)	5 (10%)	2 (4%)	3 (6%)
Pars distalis, cyst	4 (8%)	2 (4%)	3 (6%)	
Pars distalis, hyperplasia	11 (22%)	5 (10%)	6 (12%)	9 (18%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	21 (42%)	16 (32%)	18 (36%)	18 (36%)
General Body System				
Tissue, NOS	(1)	(0)	(1)	(0)
Fat, inflammation, chronic	1 (100%)			
Genital System				
Clitoral gland	(50)	(49)	(49)	(47)
Cyst	1 (2%)		1 (2%)	
Hyperplasia	4 (8%)	4 (8%)	4 (8%)	5 (11%)
Inflammation, suppurative			1 (2%)	1 (2%)
Inflammation, chronic active	8 (16%)	4 (8%)	9 (18%)	5 (11%)
Ovary	(50)	(50)	(50)	(50)
Cyst	6 (12%)	5 (10%)	6 (12%)	8 (16%)
Inflammation	1 (2%)			
Interstitial cell, hyperplasia	5 (10%)	8 (16%)	3 (6%)	6 (12%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Genital System (continued)				
Uterus	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Cyst			1 (2%)	
Decidual reaction			1 (2%)	
Dilatation			1 (2%)	
Fibrosis	1 (2%)	1 (2%)		1 (2%)
Hemorrhage	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Inflammation, chronic active				1 (2%)
Endometrium, hyperplasia, cystic	3 (6%)	3 (6%)	8 (16%)	5 (10%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Erythroid cell, hyperplasia	1 (2%)	3 (6%)	4 (8%)	1 (2%)
Lymph node	(4)	(0)	(3)	(3)
Deep cervical, angiectasis				1 (33%)
Pancreatic, angiectasis			1 (33%)	
Lymph node, bronchial	(4)	(2)	(4)	(10)
Angiectasis			1 (25%)	1 (10%)
Hyperplasia, lymphoid				2 (20%)
Lymph node, mediastinal	(29)	(27)	(30)	(25)
Angiectasis			1 (3%)	
Hyperplasia, lymphoid			1 (3%)	
Infiltration cellular, histiocyte	1 (3%)	1 (4%)		
Lymph node, mesenteric	(49)	(50)	(49)	(50)
Angiectasis	1 (2%)	1 (2%)		1 (2%)
Hemorrhage		1 (2%)		
Hyperplasia, lymphoid	2 (4%)			
Infiltration cellular, histiocyte	9 (18%)	9 (18%)	3 (6%)	4 (8%)
Spleen	(50)	(50)	(50)	(49)
Accessory spleen	1 (2%)			
Hematopoietic cell proliferation	21 (42%)	26 (52%)	22 (44%)	17 (35%)
Hemorrhage, chronic	1 (2%)			1 (2%)
Capsule, fibrosis		1 (2%)		1 (2%)
Thymus	(46)	(45)	(47)	(48)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Hyperplasia	1 (2%)			
Inflammation, chronic active	1 (2%)			
Epithelium, hyperplasia			1 (2%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)			1 (2%)
Inflammation, granulomatous				1 (2%)
Ulcer	1 (2%)	1 (2%)	1 (2%)	
Subcutaneous tissue, hemorrhage				1 (2%)
Subcutaneous tissue, inflammation	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis		1 (2%)		
Nasal, inflammation, chronic active				1 (2%)
Skeletal muscle	(2)	(0)	(1)	(0)
Fat, necrosis	1 (50%)			

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	16 ppm	32 ppm	64 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	6 (12%)	11 (22%)	4 (8%)	9 (18%)
Hemorrhage	2 (4%)			2 (4%)
Inflammation, suppurative				1 (2%)
inflammation, chronic active	1 (2%)			
Necrosis				1 (2%)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	2 (4%)	3 (6%)	2 (4%)	6 (12%)
Inflammation, chronic active		1 (2%)		3 (6%)
Metaplasia, squamous	1 (2%)			2 (4%)
Lung	(50)	(50)	(50)	(50)
Foreign body				1 (2%)
Hemorrhage			1 (2%)	
Inflammation	1 (2%)			
Inflammation, suppurative				1 (2%)
Alveolar epithelium, hyperplasia	7 (14%)	12 (24%)	13 (26%)	5 (10%)
Alveolar epithelium, metaplasia, squamous			1 (2%)	
Alveolus, infiltration cellular, histiocyte	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Nose	(49)	(49)	(50)	(50)
Foreign body	1 (2%)	4 (8%)	4 (8%)	6 (12%)
Inflammation, suppurative			1 (2%)	
Inflammation, chronic active	7 (14%)	9 (18%)	11 (22%)	18 (36%)
Epithelium, nasolacrimal duct, hyperplasia	1 (2%)			
Glands, olfactory epithelium, hyperplasia		6 (12%)	1 (2%)	2 (4%)
Glands, respiratory epithelium, hyperplasia	2 (4%)	33 (67%)	44 (88%)	47 (94%)
Nasolacrimal duct, inflammation, chronic active	3 (6%)	1 (2%)		
Olfactory epithelium, accumulation, hyaline droplet	6 (12%)	5 (10%)	6 (12%)	15 (30%)
Olfactory epithelium, atrophy	3 (6%)		28 (56%)	37 (74%)
Olfactory epithelium, degeneration			1 (2%)	4 (8%)
Olfactory epithelium, hyperplasia				1 (2%)
Olfactory epithelium, hyperplasia, basal cell		28 (57%)	42 (84%)	48 (96%)
Olfactory epithelium, metaplasia, respiratory	3 (6%)	2 (4%)	7 (14%)	17 (34%)
Olfactory epithelium, metaplasia, squamous				1 (2%)
Olfactory epithelium, necrosis			2 (4%)	5 (10%)
Respiratory epithelium, hyperplasia	2 (4%)	23 (47%)	25 (50%)	36 (72%)
Respiratory epithelium, metaplasia squamous		1 (2%)		4 (8%)
Special Senses System				
Eye	(49)	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)			
Lens, cataract	4 (8%)	5 (10%)	8 (16%)	3 (6%)
Zymbal's gland	(0)	(0)	(2)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Infarct	1 (2%)	1 (2%)		1 (2%)
Inflammation, suppurative				1 (2%)
Nephropathy, chronic	40 (80%)	46 (92%)	41 (82%)	44 (90%)
Bilateral, hydronephrosis			1 (2%)	
Pelvis, transitional epithelium, hyperplasia			1 (2%)	
Urinary bladder	(49)	(50)	(50)	(49)

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPARGYL ALCOHOL

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	8 ppm	16 ppm	32 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	9	6	6	7
Natural deaths	4		4	1
Survivors				
Died last week of study		1		
Terminal sacrifice	37	42	40	42
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(40)	(41)	(40)	(44)
Intestine large, cecum	(49)	(50)	(48)	(50)
Hemangioma				1 (2%)
Intestine small, ileum	(48)	(50)	(46)	(49)
Intestine small, jejunum	(48)	(50)	(46)	(49)
Adenoma			1 (2%)	
Carcinoma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Liver	(49)	(50)	(49)	(50)
Hemangioma			1 (2%)	
Hemangiosarcoma	1 (2%)	1 (2%)	3 (6%)	
Hepatoblastoma			1 (2%)	
Hepatocellular adenoma	10 (20%)	14 (28%)	8 (16%)	14 (28%)
Hepatocellular adenoma, multiple	11 (22%)	10 (20%)	8 (16%)	1 (2%)
Hepatocellular carcinoma	9 (18%)	8 (16%)	6 (12%)	5 (10%)
Hepatocellular carcinoma, multiple	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Hepatocholangiocarcinoma			1 (2%)	
Mesentery	(3)	(3)	(2)	(4)
Pancreas	(49)	(50)	(49)	(50)
Sarcoma, metastatic, uncertain primary site	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(49)	(50)
Mast cell tumor malignant, metastatic, uncertain primary site		1 (2%)		
Sarcoma		1 (2%)		
Squamous cell papilloma			1 (2%)	
Stomach, glandular	(49)	(50)	(49)	(50)
Sarcoma		1 (2%)		
Sarcoma, metastatic, uncertain primary site	1 (2%)			
Tooth	(3)	(4)	(5)	(2)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Sarcoma, metastatic, uncertain primary site	1 (2%)			
Endocrine System				
Adrenal cortex	(49)	(50)	(49)	(50)
Sarcoma, metastatic, uncertain primary site	1 (2%)			
Capsule, adenoma	1 (2%)			
Subcapsular, adenoma		3 (6%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Endocrine System (continued)				
Adrenal medulla	(47)	(50)	(49)	(50)
Pheochromocytoma malignant		1 (2%)		
Islets, pancreatic	(49)	(50)	(49)	(50)
Adenoma	1 (2%)			
Pituitary gland	(47)	(50)	(50)	(49)
Thyroid gland	(49)	(50)	(50)	(50)
C-cell, carcinoma		1 (2%)		
Follicular cell, adenoma			1 (2%)	
General Body System				
Peritoneum	(1)	(0)	(0)	(0)
Sarcoma, metastatic, uncertain primary site	1 (100%)			
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Hemangioma		1 (2%)		
Prostate	(50)	(50)	(50)	(49)
Seminal vesicle	(48)	(50)	(50)	(50)
Sarcoma, metastatic, uncertain primary site	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Mast cell tumor malignant, metastatic, uncertain primary site		1 (2%)		
Lymph node	(1)	(1)	(0)	(0)
Lymph node, bronchial	(37)	(35)	(30)	(33)
Hemangiosarcoma				1 (3%)
Sarcoma, metastatic, uncertain primary site	1 (3%)			
Lymph node, mandibular	(32)	(33)	(25)	(30)
Carcinoma, metastatic, Harderian gland	1 (3%)			
Mast cell tumor malignant, metastatic, uncertain primary site		1 (3%)		
Lymph node, mediastinal	(39)	(33)	(38)	(36)
Lymph node, mesenteric	(49)	(50)	(48)	(49)
Sarcoma, metastatic, uncertain primary site	1 (2%)			
Spleen	(49)	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)		1 (2%)	
Thymus	(47)	(49)	(42)	(49)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Sebaceous gland, adenoma				1 (2%)
Subcutaneous tissue, fibrous histiocytoma				2 (4%)
Subcutaneous tissue, hemangioma				1 (2%)
Subcutaneous tissue, hemangiosarcoma			1 (2%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma	1 (2%)			1 (2%)
Sarcoma				1 (2%)
Skeletal muscle	(1)	(1)	(0)	(0)
Sarcoma, metastatic, uncertain primary site	1 (100%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Harderian gland	1 (2%)			
Sarcoma, metastatic, bone				1 (2%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	9 (18%)	5 (10%)	8 (16%)	6 (12%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)		1 (2%)	1 (2%)
Alveolar/bronchiolar carcinoma	7 (14%)	6 (12%)	6 (12%)	7 (14%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)	1 (2%)	
Carcinoma, metastatic, Harderian gland	2 (4%)		1 (2%)	
Hepatoblastoma, metastatic, liver			1 (2%)	
Hepatocellular carcinoma, metastatic, liver	3 (6%)	2 (4%)	5 (10%)	2 (4%)
Bronchiole, adenoma	1 (2%)			
Nose	(49)	(50)	(50)	(50)
Hemangioma		1 (2%)		
Olfactory epithelium, sarcoma, metastatic, bone				1 (2%)
Respiratory epithelium, adenoma		1 (2%)	4 (8%)	7 (14%)
Special Senses System				
Eye	(49)	(50)	(48)	(49)
Melanoma malignant		1 (2%)		
Harderian gland	(49)	(50)	(49)	(50)
Adenoma	3 (6%)	10 (20%)	6 (12%)	9 (18%)
Carcinoma	3 (6%)	3 (6%)	5 (10%)	1 (2%)
Bilateral, adenoma				2 (4%)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Mast cell tumor malignant, metastatic, uncertain primary site				1 (2%)
Sarcoma, metastatic, uncertain primary site	1 (2%)			
Renal tubule, adenoma			1 (2%)	1 (2%)
Urinary bladder	(49)	(50)	(49)	(50)
Transitional epithelium, papilloma			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)		1 (2%)
Lymphoma malignant	1 (2%)	2 (4%)	1 (2%)	2 (4%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	38	42	40	40
Total primary neoplasms	63	76	70	69
Total animals with benign neoplasms	28	30	28	33
Total benign neoplasms	37	46	41	45
Total animals with malignant neoplasms	22	24	26	21
Total malignant neoplasms	26	30	29	24
Total animals with metastatic neoplasms	6	3	7	4
Total metastatic neoplasms	17	5	7	5
Total animals with malignant neoplasms-uncertain primary site	1	1		1

[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 C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	1/49 (2%)	3/50 (6%)	0/49 (0%)	1/50 (2%)
Adjusted rate[b]	2.3%	6.3%	0.0%	2.1%
Terminal rate[c]	1/37 (3%)	2/43 (5%)	0/40 (0%)	1/42 (2%)
First incidence (days)	729 (T)	677	—[e]	729 (T)
Poly-3 test[d]	P=0.387N	P=0.338	P=0.490N	P=0.744N
Harderian Gland: Adenoma				
Overall rate	3/50 (6%)	10/50 (20%)	6/50 (12%)	11/50 (22%)
Adjusted rate	6.8%	21.0%	12.7%	23.0%
Terminal rate	3/37 (8%)	9/43 (21%)	4/40 (10%)	9/42 (21%)
First incidence (days)	729 (T)	677	630	505
Poly-3 test	P=0.062	P=0.046	P=0.273	P=0.027
Harderian Gland: Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	5/50 (10%)	1/50 (2%)
Adjusted rate	6.7%	6.3%	10.7%	2.1%
Terminal rate	2/37 (5%)	3/43 (7%)	5/40 (13%)	1/42 (2%)
First incidence (days)	711	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.254N	P=0.632N	P=0.381	P=0.287N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	13/50 (26%)	11/50 (22%)	11/50 (22%)
Adjusted rate	13.5%	27.3%	23.3%	23.0%
Terminal rate	5/37 (14%)	12/43 (28%)	9/40 (23%)	9/42 (21%)
First incidence (days)	711	677	630	505
Poly-3 test	P=0.287	P=0.082	P=0.173	P=0.180
Liver: Hemangiosarcoma				
Overall rate	1/49 (2%)	1/50 (2%)	3/49 (6%)	0/50 (0%)
Adjusted rate	2.3%	2.1%	6.5%	0.0%
Terminal rate	1/37 (3%)	1/43 (2%)	2/40 (5%)	0/42 (0%)
First incidence (days)	729 (T)	729 (T)	698	—
Poly-3 test	P=0.389N	P=0.742N	P=0.325	P=0.486N
Liver: Hepatocellular Adenoma				
Overall rate	21/49 (43%)	24/50 (48%)	16/49 (33%)	15/50 (30%)
Adjusted rate	45.4%	49.8%	34.1%	31.1%
Terminal rate	16/37 (43%)	21/43 (49%)	14/40 (35%)	11/42 (26%)
First incidence (days)	440	572	493	499
Poly-3 test	P=0.041N	P=0.412	P=0.181N	P=0.111N
Liver: Hepatocellular Carcinoma				
Overall rate	10/49 (20%)	9/50 (18%)	8/49 (16%)	7/50 (14%)
Adjusted rate	21.7%	18.8%	17.2%	14.6%
Terminal rate	5/37 (14%)	6/43 (14%)	5/40 (13%)	4/42 (10%)
First incidence (days)	440	677	638	499
Poly-3 test	P=0.220N	P=0.466N	P=0.388N	P=0.265N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	26/49 (53%)	31/50 (62%)	22/49 (45%)	19/50 (38%)
Adjusted rate	54.8%	64.2%	46.2%	39.2%
Terminal rate	18/37 (49%)	27/43 (63%)	17/40 (43%)	14/42 (33%)
First incidence (days)	440	572	493	499
Poly-3 test	P=0.022N	P=0.233	P=0.262N	P=0.090N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	10/49 (20%)	9/50 (18%)	9/49 (18%)	7/50 (14%)
Adjusted rate	21.7%	18.8%	19.1%	14.6%
Terminal rate	5/37 (14%)	6/43 (14%)	5/40 (13%)	4/42 (10%)
First incidence (days)	440	677	565	499
Poly-3 test	P=0.230N	P=0.466N	P=0.479N	P=0.265N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	26/49 (53%)	31/50 (62%)	23/49 (47%)	19/50 (38%)
Adjusted rate	54.8%	64.2%	47.7%	39.2%
Terminal rate	18/37 (49%)	27/43 (63%)	17/40 (43%)	14/42 (33%)
First incidence (days)	440	572	493	499
Poly-3 test	P=0.023N	P=0.233	P=0.313N	P=0.090N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	10/50 (20%)	5/50 (10%)	9/50 (18%)	7/50 (14%)
Adjusted rate	22.4%	10.5%	19.1%	14.9%
Terminal rate	8/37 (22%)	5/43 (12%)	8/40 (20%)	7/42 (17%)
First incidence (days)	688	729 (T)	565	729 (T)
Poly-3 test	P=0.363N	P=0.103N	P=0.449N	P=0.259N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	7/50 (14%)	7/50 (14%)	7/50 (14%)	7/50 (14%)
Adjusted rate	15.5%	14.8%	15.0%	14.9%
Terminal rate	6/37 (16%)	7/43 (16%)	7/40 (18%)	7/42 (17%)
First incidence (days)	509	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.535N	P=0.574N	P=0.589N	P=0.584N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	17/50 (34%)	10/50 (20%)	14/50 (28%)	13/50 (26%)
Adjusted rate	37.5%	21.1%	29.7%	27.7%
Terminal rate	14/37 (38%)	10/43 (23%)	13/40 (33%)	13/42 (31%)
First incidence (days)	509	729 (T)	565	729 (T)
Poly-3 test	P=0.333N	P=0.063N	P=0.283N	P=0.217N
Nose: Adenoma				
Overall rate	0/49 (0%)	1/50 (2%)	4/50 (8%)	7/50 (14%)
Adjusted rate	0.0%	2.1%	8.6%	14.9%
Terminal rate	0/37 (0%)	1/43 (2%)	3/40 (8%)	7/42 (17%)
First incidence (days)	—	729 (T)	698	729 (T)
Poly-3 test	P<0.001	P=0.517	P=0.069	P=0.010
All Organs: Hemangiosarcoma				
Overall rate	2/50 (4%)	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rate	4.5%	2.1%	8.5%	2.1%
Terminal rate	2/37 (5%)	1/43 (2%)	2/40 (5%)	1/42 (2%)
First incidence (days)	729 (T)	729 (T)	631	729 (T)
Poly-3 test	P=0.485N	P=0.477N	P=0.364	P=0.482N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	5/50 (10%)	3/50 (6%)
Adjusted rate	4.5%	6.3%	10.6%	6.4%
Terminal rate	2/37 (5%)	3/43 (7%)	3/40 (8%)	3/42 (7%)
First incidence (days)	729 (T)	729 (T)	631	729 (T)
Poly-3 test	P=0.431	P=0.530	P=0.240	P=0.524

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
All Organs: Benign Neoplasms				
Overall rate	28/50 (56%)	30/50 (60%)	28/50 (56%)	33/50 (66%)
Adjusted rate	59.1%	62.0%	57.3%	67.3%
Terminal rate	21/37 (57%)	26/43 (61%)	22/40 (55%)	27/42 (64%)
First incidence (days)	440	572	493	499
Poly-3 test	P=0.252	P=0.470	P=0.508N	P=0.267
All Organs: Malignant Neoplasms				
Overall rate	22/50 (44%)	24/50 (48%)	26/50 (52%)	22/50 (44%)
Adjusted rate	46.1%	50.2%	53.5%	44.6%
Terminal rate	15/37 (41%)	21/43 (49%)	19/40 (48%)	15/42 (36%)
First incidence (days)	440	677	565	499
Poly-3 test	P=0.446N	P=0.420	P=0.299	P=0.523N
All Organs: Benign or Malignant Neoplasms				
Overall rate	38/50 (76%)	42/50 (84%)	40/50 (80%)	40/50 (80%)
Adjusted rate	77.2%	86.6%	80.0%	80.0%
Terminal rate	27/37 (73%)	37/43 (86%)	30/40 (75%)	32/42 (76%)
First incidence (days)	440	572	493	499
Poly-3 test	P=0.549	P=0.170	P=0.462	P=0.462

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal cortex, liver, lung, and nose; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE C3
Historical Incidence of Harderian Gland Adenoma in Untreated Male B6C3F1 Mice[a]

Study	Incidence in Controls
Historical Incidence: Inhalation Studies	
Cumene	8/50
Decalin	6/50
Divinylbenzene	5/50
Methyl isobutyl ketone	5/50
α-Methylstyrene	10/50
Propargyl alcohol	3/50
Propylene glycol mono-*t*-butyl ether	6/50
Stoddard solvent (Type IIC)	7/50
Vanadium pentoxide	8/50
Overall Historical Incidence: Inhalation Studies	
Total (%)	58/450 (12.9%)
Mean ± standard deviation	12.9% ± 4.1%
Range	6%-20%
Overall Historical Incidence: All Routes	
Total (%)	185/1,449 (12.3%)
Mean ± standard deviation	12.5% ± 4.2%
Range	4%-22%

[a] Data as of March 5, 2007

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	8 ppm	16 ppm	32 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	9	6	6	7
Natural deaths	4		4	1
Survivors				
Died last week of study		1		
Terminal sacrifice	37	42	40	42
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(40)	(41)	(40)	(44)
Hyperplasia	1 (3%)			
Intestine large, cecum	(49)	(50)	(48)	(50)
Intestine small, ileum	(48)	(50)	(46)	(49)
Infiltration cellular, mixed cell		1 (2%)		
Intestine small, jejunum	(48)	(50)	(46)	(49)
Peyer's patch, hyperplasia		1 (2%)		
Liver	(49)	(50)	(49)	(50)
Angiectasis				1 (2%)
Basophilic focus	5 (10%)	8 (16%)	5 (10%)	5 (10%)
Clear cell focus	18 (37%)	11 (22%)	13 (27%)	4 (8%)
Eosinophilic focus	3 (6%)	3 (6%)	2 (4%)	3 (6%)
Hyperplasia, regenerative	1 (2%)			
Infarct	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Inflammation, chronic	1 (2%)	1 (2%)		
Mixed cell focus	1 (2%)		1 (2%)	1 (2%)
Necrosis	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Tension lipidosis	1 (2%)	4 (8%)	3 (6%)	3 (6%)
Thrombosis	1 (2%)			
Vacuolization cytoplasmic, focal			1 (2%)	
Bile duct, hyperplasia, adenomatous		1 (2%)		
Mesentery	(3)	(3)	(2)	(4)
Fat, necrosis	3 (100%)	3 (100%)	2 (100%)	4 (100%)
Pancreas	(49)	(50)	(49)	(50)
Atrophy	1 (2%)		2 (4%)	2 (4%)
Basophilic focus				1 (2%)
Duct, cyst	1 (2%)		1 (2%)	
Duct, hyperplasia, adenomatous		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Stomach, forestomach	(50)	(50)	(49)	(50)
Hyperplasia, squamous	3 (6%)	1 (2%)	2 (4%)	6 (12%)
Inflammation, chronic active	2 (4%)		2 (4%)	4 (8%)
Ulcer		1 (2%)		
Stomach, glandular	(49)	(50)	(49)	(50)
Infiltration cellular, mixed cell		1 (2%)		
Mineralization			2 (4%)	
Tooth	(3)	(4)	(5)	(2)
Inflammation, chronic active			1 (20%)	
Malformation	1 (33%)			

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

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	5 (10%)	9 (18%)	8 (16%)	9 (18%)
Inflammation, suppurative			1 (2%)	
Thrombosis		1 (2%)	1 (2%)	
Endocrine System				
Adrenal cortex	(49)	(50)	(49)	(50)
Hyperplasia	12 (24%)	16 (32%)	9 (18%)	13 (26%)
Hypertrophy	24 (49%)	24 (48%)	22 (45%)	18 (36%)
Subcapsular, hyperplasia	1 (2%)			
Adrenal medulla	(47)	(50)	(49)	(50)
Hyperplasia	2 (4%)	3 (6%)		3 (6%)
Islets, pancreatic	(49)	(50)	(49)	(50)
Hyperplasia		2 (4%)		
Pituitary gland	(47)	(50)	(50)	(49)
Pars distalis, hyperplasia	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Thyroid gland	(49)	(50)	(50)	(50)
Follicular cell, hyperplasia	17 (35%)	22 (44%)	14 (28%)	8 (16%)
General Body System				
Peritoneum	(1)	(0)	(0)	(0)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Granuloma sperm		1 (2%)	1 (2%)	1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Abscess		1 (2%)		
Inflammation, chronic active			1 (2%)	2 (4%)
Prostate	(50)	(50)	(50)	(49)
Inflammation, suppurative		3 (6%)	1 (2%)	
Seminal vesicle	(48)	(50)	(50)	(50)
Inflammation, suppurative		2 (4%)	1 (2%)	
Testes	(50)	(50)	(50)	(50)
Atrophy		1 (2%)	1 (2%)	1 (2%)
Germinal epithelium, degeneration		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)		1 (2%)	1 (2%)
Lymph node	(1)	(1)	(0)	(0)
Lymph node, bronchial	(37)	(35)	(30)	(33)
Lymph node, mandibular	(32)	(33)	(25)	(30)
Lymph node, mediastinal	(39)	(33)	(38)	(36)
Lymph node, mesenteric	(49)	(50)	(48)	(49)
Hyperplasia, lymphoid	1 (2%)			
Infiltration cellular, mixed cell		1 (2%)		
Necrosis	1 (2%)			
Spleen	(49)	(50)	(49)	(50)
Hematopoietic cell proliferation		4 (8%)	2 (4%)	
Thymus	(47)	(49)	(42)	(49)
Cyst				1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Edema		1 (2%)		
Inflammation, chronic active	8 (16%)	3 (6%)	3 (6%)	5 (10%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fracture		1 (2%)		
Hyperostosis		1 (2%)		
Skeletal muscle	(1)	(1)	(0)	(0)
Inflammation, suppurative		1 (100%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Inflammation, chronic active		2 (4%)		
Metaplasia, squamous		1 (2%)		
Thrombosis	1 (2%)			
Alveolar epithelium, hyperplasia	4 (8%)	4 (8%)	5 (10%)	7 (14%)
Alveolus, infiltration cellular, histiocyte	4 (8%)	2 (4%)	2 (4%)	2 (4%)
Bronchiole, goblet cell, hyperplasia		1 (2%)		
Bronchiole, hyperplasia		1 (2%)	2 (4%)	
Nose	(49)	(50)	(50)	(50)
Inflammation, suppurative	2 (4%)	16 (32%)	25 (50%)	50 (100%)
Glands, respiratory epithelium, hyperplasia	17 (35%)	29 (58%)	40 (80%)	50 (100%)
Glands, necrosis			1 (2%)	
Olfactory epithelium, atrophy		3 (6%)	21 (42%)	33 (66%)
Olfactory epithelium, metaplasia, respiratory	5 (10%)		7 (14%)	16 (32%)
Olfactory epithelium, necrosis		1 (2%)		
Respiratory epithelium, hyperplasia	1 (2%)	49 (98%)	49 (98%)	50 (100%)
Respiratory epithelium, metaplasia, squamous	2 (4%)	11 (22%)	36 (72%)	50 (100%)
Respiratory epithelium, necrosis			2 (4%)	
Turbinate, atrophy		50 (100%)	49 (98%)	50 (100%)
Special Senses System				
Eye	(49)	(50)	(48)	(49)
Cataract			1 (2%)	2 (4%)
Degeneration		1 (2%)		
Phthisis bulbi	1 (2%)			
Cornea, epithelium, hyperplasia			2 (4%)	
Cornea, fibrosis	2 (4%)			2 (4%)
Cornea, inflammation, chronic active	1 (2%)		5 (10%)	7 (14%)
Cornea, ulcer	1 (2%)			1 (2%)
Harderian gland	(49)	(50)	(49)	(50)
Hyperplasia	2 (4%)	5 (10%)	2 (4%)	3 (6%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Cyst		1 (2%)	2 (4%)	
Inflammation, suppurative	1 (2%)	2 (4%)	1 (2%)	
Metaplasia, osseous	3 (6%)	3 (6%)	4 (8%)	5 (10%)
Nephropathy	46 (94%)	47 (94%)	47 (94%)	46 (92%)
Artery, inflammation, chronic		1 (2%)		
Capsule, inflammation, chronic				1 (2%)
Medulla, necrosis	1 (2%)			
Pelvis, dilatation		2 (4%)		
Renal tubule, hyperplasia			1 (2%)	1 (2%)
Urinary bladder	(49)	(50)	(49)	(50)
Inflammation, suppurative	1 (2%)	2 (4%)		

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPARGYL ALCOHOL

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	8 ppm	16 ppm	32 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	8	15	10
Natural deaths	2	3	3	2
Survivors				
Terminal sacrifice	39	39	32	38
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(41)	(40)	(45)	(44)
Intestine large, cecum	(48)	(48)	(47)	(49)
Intestine large, rectum	(49)	(48)	(48)	(49)
Intestine small, duodenum	(49)	(47)	(48)	(48)
Carcinoma			1 (2%)	
Intestine small, ileum	(48)	(47)	(47)	(49)
Intestine small, jejunum	(48)	(47)	(47)	(48)
Carcinoma		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)			
Hemangioma		1 (2%)		
Hemangiosarcoma				1 (2%)
Hepatocellular adenoma	12 (24%)	7 (14%)	9 (18%)	4 (8%)
Hepatocellular adenoma, multiple	3 (6%)	7 (14%)	3 (6%)	
Hepatocellular carcinoma	4 (8%)	6 (12%)		
Hepatocholangiocarcinoma			1 (2%)	
Osteosarcoma, metastatic, bone				1 (2%)
Mesentery	(9)	(13)	(7)	(5)
Hepatocholangiocarcinoma, metastatic, liver			1 (14%)	
Osteosarcoma, metastatic, bone				1 (20%)
Pancreas	(50)	(48)	(50)	(49)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Sarcoma, metastatic, skin				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)			
Stomach, glandular	(50)	(48)	(48)	(49)
Tongue	(1)	(1)	(0)	(0)
Squamous cell carcinoma		1 (100%)		
Squamous cell papilloma	1 (100%)			
Tooth	(0)	(0)	(1)	(0)
Cardiovascular System				
Blood vessel	(1)	(0)	(0)	(0)
Aorta, sarcoma, metastatic, skin	1 (100%)			
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	1 (2%)
Sarcoma, metastatic, skin	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Capsule, adenoma		1 (2%)		
Capsule, carcinoma			1 (2%)	
Adrenal medulla	(48)	(46)	(50)	(50)
Pheochromocytoma benign	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Pheochromocytoma malignant				1 (2%)
Islets, pancreatic	(50)	(48)	(50)	(49)
Carcinoma	1 (2%)			
Pituitary gland	(50)	(49)	(48)	(50)
Pars distalis, adenoma	4 (8%)	7 (14%)	7 (15%)	5 (10%)
Pars distalis, carcinoma	1 (2%)			
Pars intermedia, adenoma				1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	2 (4%)	3 (6%)	1 (2%)	
General Body System				
Tissue NOS	(1)	(0)	(0)	(0)
Carcinoma	1 (100%)			
Genital System				
Ovary	(49)	(50)	(49)	(49)
Cystadenoma	6 (12%)	3 (6%)		
Granulosa cell tumor benign		1 (2%)	1 (2%)	
Hemangiosarcoma			1 (2%)	
Leiomyosarcoma	1 (2%)			
Luteoma		2 (4%)		
Osteosarcoma, metastatic, bone				1 (2%)
Tubulostromal adenoma		1 (2%)		1 (2%)
Uterus	(50)	(50)	(50)	(49)
Carcinoma			1 (2%)	
Hemangiosarcoma			4 (8%)	
Leiomyosarcoma	1 (2%)			
Polyp stromal	2 (4%)	1 (2%)	3 6%)	2 (4%)
Sarcoma stromal				1 (2%)
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(50)
Hemangiosarcoma	2 (4%)			
Lymph node	(9)	(4)	(5)	(4)
Sarcoma, metastatic, skin			1 (20%)	
Lumbar, hemangiosarcoma		1 (25%)		
Lymph node, bronchial	(42)	(40)	(38)	(32)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (3%)	
Lymph node, mandibular	(38)	(33)	(38)	(39)
Lymph node, mediastinal	(45)	(38)	(42)	(33)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Sarcoma, metastatic, skin	1 (2%)		1 (2%)	
Lymph node, mesenteric	(48)	(47)	(49)	(49)
Sarcoma, metastatic, skin			1 (2%)	

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Hematopoietic System (continued)				
Spleen	(50)	(48)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Osteosarcoma, metastatic, bone				1 (2%)
Thymus	(49)	(47)	(48)	(50)
Alveolar/bronchiolar carcinoma, metastatic,			1 (2%)	
Sarcoma, metastatic, skin	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(48)
Carcinoma				2 (4%)
Fibroadenoma			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrous histiocytoma, multiple		1 (2%)		
Subcutaneous tissue, liposarcoma		1 (2%)		
Subcutaneous tissue, sarcoma	2 (4%)		2 (4%)	2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma				1 (2%)
Sarcoma		1 (2%)		
Skeletal muscle	(1)	(1)	(2)	(0)
Hemangiosarcoma		1 (100%)		
Hepatocholangiocarcinoma, metastatic, liver			1 (50%)	
Sarcoma, metastatic, skin	1 (100%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland	1 (2%)			
Spinal cord	(0)	(0)	(0)	(1)
Osteosarcoma, metastatic, bone				1 (100%)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	6 (12%)	2 (4%)	3 (6%)
Alveolar/bronchiolar carcinoma	2 (4%)	3 (6%)		1 (2%)
Alveolar/bronchiolar carcinoma, multiple			2 (4%)	1 (2%)
Carcinoma, metastatic, Harderian gland				1 (2%)
Hemangiosarcoma, metastatic, uterus			1 (2%)	
Hepatocellular carcinoma, metastatic, liver	3 (6%)	1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Liposarcoma, metastatic, skin		1 (2%)		
Osteosarcoma, metastatic, bone				1 (2%)
Sarcoma, metastatic, skin	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Respiratory epithelium, adenoma		2 (4%)	4 (8%)	6 (12%)
Pleura	(1)	(0)	(0)	(0)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (100%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Special Senses System				
Eye	(49)	(48)	(49)	(48)
Melanoma benign		1 (2%)		
Harderian gland	(50)	(49)	(50)	(50)
Adenoma	4 (8%)	2 (4%)	5 (10%)	3 (6%)
Carcinoma		1 (2%)	1 (2%)	2 (4%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Osteosarcoma, metastatic, bone				1 (2%)
Transitional epithelium, carcinoma				1 (2%)
Urinary bladder	(50)	(47)	(48)	(49)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Lymphoma malignant	13 (26%)	8 (16%)	7 (14%)	6 (12%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	37	42	42	34
Total primary neoplasms	69	76	62	50
Total animals with benign neoplasms	26	31	32	20
Total benign neoplasms	39	47	37	26
Total animals with malignant neoplasms	23	24	23	19
Total malignant neoplasms	30	29	25	24
Total animals with metastatic neoplasms	7	2	4	3
Total metastatic neoplasms	13	2	11	9

[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 D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Harderian Gland: Adenoma				
Overall rate[a]	4/50 (8%)	2/50 (4%)	5/50 (10%)	3/50 (6%)
Adjusted rate[b]	8.8%	4.3%	11.6%	6.9%
Terminal rate[c]	4/39 (10%)	2/39 (5%)	5/32 (16%)	3/38 (8%)
First incidence (days)	731 (T)	731 (T)	731 (T)	731 (T)
Poly-3 test[d]	P=0.567N	P=0.330N	P=0.468	P=0.521N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	6/50 (12%)	5/50 (10%)
Adjusted rate	8.8%	6.5%	13.9%	11.4%
Terminal rate	4/39 (10%)	3/39 (8%)	6/32 (19%)	5/38 (13%)
First incidence (days)	731 (T)	731 (T)	731 (T)	731 (T)
Poly-3 test	P=0.305	P=0.492N	P=0.337	P=0.476
Liver: Hepatocellular Adenoma				
Overall rate	15/50 (30%)	14/50 (28%)	12/50 (24%)	4/50 (8%)
Adjusted rate	32.8%	30.1%	27.1%	9.2%
Terminal rate	13/39 (33%)	13/39 (33%)	8/32 (25%)	4/38 (11%)
First incidence (days)	647	605	436	731 (T)
Poly-3 test	P=0.004N	P=0.479N	P=0.362N	P=0.005N
Liver: Hepatocellular Carcinoma				
Overall rate	4/50 (8%)	6/50 (12%)	0/50 (0%)	0/50 (0%)
Adjusted rate	8.8%	13.0%	0.0%	0.0%
Terminal rate	4/39 (10%)	5/39 (13%)	0/32 (0%)	0/38 (0%)
First incidence (days)	731 (T)	712	—[e]	—
Poly-3 test	P=0.011N	P=0.380	P=0.067N	P=0.065N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	17/50 (34%)	19/50 (38%)	12/50 (24%)	4/50 (8%)
Adjusted rate	37.1%	40.8%	27.1%	9.2%
Terminal rate	15/39 (39%)	17/39 (44%)	8/32 (25%)	4/38 (11%)
First incidence (days)	647	605	436	731 (T)
Poly-3 test	P<0.001N	P=0.442	P=0.213N	P<0.001N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/50 (6%)	6/50 (12%)	2/50 (4%)	3/50 (6%)
Adjusted rate	6.6%	12.8%	4.6%	6.9%
Terminal rate	3/39 (8%)	5/39 (13%)	2/32 (6%)	3/38 (8%)
First incidence (days)	731 (T)	495	731 (T)	731 (T)
Poly-3 test	P=0.416N	P=0.257	P=0.523N	P=0.645
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	4.3%	6.5%	4.6%	4.6%
Terminal rate	1/39 (3%)	3/39 (8%)	1/32 (3%)	2/38 (5%)
First incidence (days)	389	731 (T)	718	731 (T)
Poly-3 test	P=0.552N	P=0.499	P=0.669	P=0.673
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	5/50 (10%)	8/50 (16%)	4/50 (8%)	5/50 (10%)
Adjusted rate	10.8%	17.1%	9.3%	11.4%
Terminal rate	4/39 (10%)	7/39 (18%)	3/32 (9%)	5/38 (13%)
First incidence (days)	389	495	718	731 (T)
Poly-3 test	P=0.459N	P=0.283	P=0.543N	P=0.594

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Nose: Adenoma				
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)	6/50 (12%)
Adjusted rate	0.0%	4.3%	9.3%	13.7%
Terminal rate	0/39 (0%)	2/39 (5%)	3/32 (9%)	6/38 (16%)
First incidence (days)	—	731 (T)	722	731 (T)
Poly-3 test	P=0.006	P=0.241	P=0.054	P=0.013
Ovary: Cystadenoma				
Overall rate	6/49 (12%)	3/50 (6%)	0/49 (0%)	0/49 (0%)
Adjusted rate	13.2%	6.4%	0.0%	0.0%
Terminal rate	5/39 (13%)	2/39 (5%)	0/31 (0%)	0/37 (0%)
First incidence (days)	389	605	—	—
Poly-3 test	P=0.003N	P=0.232N	P=0.020N	P=0.019N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	4/50 (8%)	7/49 (14%)	7/48 (15%)	5/50 (10%)
Adjusted rate	8.7%	15.4%	16.3%	11.4%
Terminal rate	2/39 (5%)	6/39 (15%)	3/31 (10%)	4/38 (11%)
First incidence (days)	647	712	509	718
Poly-3 test	P=0.469	P=0.258	P=0.225	P=0.471
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	5/50 (10%)	7/49 (14%)	7/48 (15%)	5/50 (10%)
Adjusted rate	10.8%	15.4%	16.3%	11.4%
Terminal rate	2/39 (5%)	6/39 (15%)	3/31 (10%)	4/38 (11%)
First incidence (days)	592	712	509	718
Poly-3 test	P=0.551N	P=0.368	P=0.328	P=0.594
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	4.4%	6.5%	2.3%	0.0%
Terminal rate	1/39 (3%)	3/39 (8%)	1/32 (3%)	0/38 (0%)
First incidence (days)	647	731 (T)	731 (T)	—
Poly-3 test	P=0.113N	P=0.504	P=0.520N	P=0.247N
Uterus: Stromal Polyp				
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	4.4%	2.2%	6.9%	4.6%
Terminal rate	2/39 (5%)	1/39 (3%)	2/32 (6%)	2/38 (5%)
First incidence (days)	731 (T)	731 (T)	544	731 (T)
Poly-3 test	P=0.475	P=0.495N	P=0.483	P=0.680
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	4.4%	2.2%	6.9%	6.8%
Terminal rate	2/39 (5%)	1/39 (3%)	2/32 (6%)	2/38 (5%)
First incidence (days)	731 (T)	731 (T)	544	677
Poly-3 test	P=0.283	P=0.495N	P=0.483	P=0.485
Uterus: Hemangiosarcoma				
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	9.2%	0.0%
Terminal rate	0/39 (0%)	0/39 (0%)	2/32 (6%)	0/38 (0%)
First incidence (days)	—	—	620	—
Poly-3 test	P=0.471	—[f]	P=0.055	—

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
All Organs: Hemangiosarcoma				
Overall rate	2/50 (4%)	2/50 (4%)	5/50 (10%)	1/50 (2%)
Adjusted rate	4.4%	4.3%	11.3%	2.2%
Terminal rate	2/39 (5%)	2/39 (5%)	2/32 (6%)	0/38 (0%)
First incidence (days)	731 (T)	731 (T)	467	388
Poly-3 test	P=0.472N	P=0.688N	P=0.207	P=0.507N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	5/50 (10%)	1/50 (2%)
Adjusted rate	4.4%	6.5%	11.3%	2.2%
Terminal rate	2/39 (5%)	3/39 (8%)	2/32 (6%)	0/38 (0%)
First incidence (days)	731 (T)	731 (T)	467	388
Poly-3 test	P=0.412N	P=0.507	P=0.207	P=0.507N
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	2.2%	6.3%	4.5%	4.5%
Terminal rate	0/39 (0%)	0/39 (0%)	0/32 (0%)	0/38 (0%)
First incidence (days)	716	495	436	642
Poly-3 test	P=0.481	P=0.322	P=0.490	P=0.491
All Organs: Malignant Lymphoma				
Overall rate	13/50 (26%)	8/50 (16%)	7/50 (14%)	6/50 (12%)
Adjusted rate	28.2%	16.9%	15.7%	13.6%
Terminal rate	11/39 (28%)	5/39 (13%)	4/32 (13%)	5/38 (13%)
First incidence (days)	579	558	579	626
Poly-3 test	P=0.069N	P=0.142N	P=0.117N	P=0.073N
All Organs: Benign Neoplasms				
Overall rate	26/50 (52%)	31/50 (62%)	32/50 (64%)	20/50 (40%)
Adjusted rate	55.6%	65.5%	67.8%	45.7%
Terminal rate	22/39 (56%)	28/39 (72%)	22/32 (69%)	19/38 (50%)
First incidence (days)	389	495	436	718
Poly-3 test	P=0.145N	P=0.216	P=0.153	P=0.230N
All Organs: Malignant Neoplasms				
Overall rate	23/50 (46%)	24/50 (48%)	23/50 (46%)	19/50 (38%)
Adjusted rate	47.5%	48.9%	47.6%	41.0%
Terminal rate	16/39 (41%)	16/39 (41%)	10/32 (31%)	12/38 (32%)
First incidence (days)	369	495	436	388
Poly-3 test	P=0.267N	P=0.525	P=0.578	P=0.333N
All Organs: Benign or Malignant Neoplasms				
Overall rate	37/50 (74%)	42/50 (84%)	42/50 (84%)	34/50 (68%)
Adjusted rate	75.9%	84.9%	84.4%	73.3%
Terminal rate	28/39 (72%)	33/39 (85%)	25/32 (78%)	26/38 (68%)
First incidence (days)	369	495	436	388
Poly-3 test	P=0.330N	P=0.188	P=0.206	P=0.478N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, nose, ovary, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	8 ppm	16 ppm	32 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	8	15	10
Natural deaths	2	3	3	2
Survivors				
Terminal sacrifice	39	39	32	38
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Epithelium, hyperplasia			1 (2%)	
Gallbladder	(41)	(40)	(45)	(44)
Intestine large, cecum	(48)	(48)	(47)	(49)
Intestine large, rectum	(49)	(48)	(48)	(49)
Artery, inflammation, chronic active		1 (2%)		
Intestine small, duodenum	(49)	(47)	(48)	(48)
Peyer's patch, hyperplasia				1 (2%)
Intestine small, ileum	(48)	(47)	(47)	(49)
Infiltration, cellular, eosinophil		1 (2%)		
Intestine small, jejunum	(48)	(47)	(47)	(48)
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		1 (2%)
Basophilic focus	1 (2%)	3 (6%)	2 (4%)	3 (6%)
Clear cell focus	2 (4%)	3 (6%)	3 (6%)	2 (4%)
Eosinophilic focus	6 (12%)	7 (14%)	3 (6%)	
Hematopoietic cell proliferation	2 (4%)		2 (4%)	1 (2%)
Infiltration cellular, mononuclear cell			1 (2%)	
Necrosis	2 (4%)		2 (4%)	2 (4%)
Tension lipidosis	2 (4%)	1 (2%)	5 (10%)	2 (4%)
Bile duct, hyperplasia				1 (2%)
Mesentery	(9)	(13)	(7)	(5)
Inflammation, chronic active		1 (8%)		1 (20%)
Artery, inflammation, chronic active	1 (11%)			
Fat, hemorrhage		1 (8%)		
Fat, necrosis	8 (89%)	11 (85%)	5 (71%)	4 (80%)
Pancreas	(50)	(48)	(50)	(49)
Amyloid deposition				1 (2%)
Atrophy	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Basophilic focus		1 (2%)		
Infiltration cellular, mononuclear cell			1 (2%)	
Inflammation, acute		1 (2%)		
Acinus, degeneration		1 (2%)		
Artery, inflammation, chronic active		1 (2%)		
Duct, cyst		1 (2%)		1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, squamous	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Infiltration cellular, mast cell			1 (2%)	
Inflammation, chronic active	1 (2%)		2 (4%)	
Ulcer	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Artery, inflammation, chronic active				1 (2%)

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

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Alimentary System (continued)				
Stomach, glandular	(50)	(48)	(48)	(49)
Infiltration cellular, mixed cell	1 (2%)			1 (2%)
Inflammation, acute				1 (2%)
Necrosis		1 (2%)		
Tongue	(1)	(1)	(0)	(0)
Tooth	(0)	(0)	(1)	(0)
Cardiovascular System				
Blood vessel	(1)	(0)	(0)	(0)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	2 (4%)	5 (10%)	7 (14%)	3 (6%)
Inflammation, suppurative			1 (2%)	
Necrosis				1 (2%)
Thrombosis			1 (2%)	1 (2%)
Artery, inflammation, chronic active	1 (2%)	2 (4%)		1 (2%)
Capillary, hyperplasia			1 (2%)	
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Hematopoietic cell proliferation		1 (2%)		
Hyperplasia	6 (12%)	2 (4%)	2 (4%)	5 (10%)
Hypertrophy	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Necrosis			1 (2%)	
Thrombosis			1 (2%)	
Capsule, hyperplasia				1 (2%)
Subcapsular, hyperplasia	1 (2%)			
Adrenal medulla	(48)	(46)	(50)	(50)
Hyperplasia	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Islets, pancreatic	(50)	(48)	(50)	(49)
Hyperplasia	1 (2%)	1 (2%)		
Pituitary gland	(50)	(49)	(48)	(50)
Cyst, multiple				1 (2%)
Pars distalis, angiectasis	5 (10%)	2 (4%)		3 (6%)
Pars distalis, hyperplasia	23 (46%)	11 (22%)	14 (29%)	9 (18%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, hyperplasia	11 (22%)	13 (26%)	14 (28%)	11 (22%)
General Body System				
Tissue NOS	(1)	(0)	(0)	(0)
Genital System				
Ovary	(49)	(50)	(49)	(49)
Angiectasis	1 (2%)	1 (2%)		1 (2%)
Cyst	10 (20%)	13 (26%)	15 (31%)	9 (18%)
Thrombosis		2 (4%)		
Uterus	(50)	(50)	(50)	(49)
Amyloid deposition	1 (2%)			
Angiectasis	2 (4%)		2 (4%)	3 (6%)
Inflammation, suppurative		1 (2%)		
Necrosis		1 (2%)		
Endometrium, hyperplasia, cystic	6 (12%)	4 (8%)	1 (2%)	

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(50)
Hyperplasia, reticulum cell	1 (2%)		1 (2%)	
Lymph node	(9)	(4)	(5)	(4)
Deep cervical, ectasia			1 (20%)	
Iliac, hemorrhage	1 (11%)			
Renal, ectasia		1 (25%)		
Lymph node, bronchial	(42)	(40)	(38)	(32)
Lymph node, mandibular	(38)	(33)	(38)	(39)
Lymph node, mediastinal	(45)	(38)	(42)	(33)
Lymph node, mesenteric	(48)	(47)	(49)	(49)
Infiltration cellular, eosinophil		1 (2%)		
Spleen	(50)	(48)	(50)	(50)
Hematopoietic cell proliferation	3 (6%)	4 (8%)	3 (6%)	4 (8%)
Hyperplasia, lymphoid			1 (2%)	
Thymus	(49)	(47)	(48)	(50)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(48)
Hyperplasia			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion				1 (2%)
Inflammation, acute		1 (2%)	1 (2%)	
Inflammation, chronic active		2 (4%)	1 (2%)	1 (2%)
Ulcer	1 (2%)			
Subcutaneous tissue, edema		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)	(1)	(2)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Inflammation, suppurative			1 (2%)	
Artery, inflammation, chronic active				1 (2%)
Meninges, infiltration cellular, mononuclear cell	1 (2%)			
Spinal cord	(0)	(0)	(0)	(1)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Artery, inflammation, chronic active				1 (2%)
Lung	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Infiltration cellular, polymorphonuclear			1 (2%)	
Inflammation, granulomatous	1 (2%)			
Metaplasia, squamous			1 (2%)	
Alveolar epithelium, hyperplasia	7 (14%)	6 (12%)	3 (6%)	4 (8%)
Alveolus, infiltration cellular, histiocyte	1 (2%)	1 (2%)	2 (4%)	2 (4%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Propargyl Alcohol

	Chamber Control	8 ppm	16 ppm	32 ppm
Respiratory System (continued)				
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)	4 (8%)	35 (70%)	45 (90%)
Polyp, inflammatory				1 (2%)
Glands, respiratory epithelium, hyperplasia	7 (14%)	24 (48%)	44 (88%)	49 (98%)
Olfactory epithelium, atrophy	2 (4%)	5 (10%)	31 (62%)	29 (58%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)		6 (12%)	14 (28%)
Olfactory epithelium, necrosis			1 (2%)	1 (2%)
Respiratory epithelium, hyperplasia		50 (100%)	50 (100%)	49 (98%)
Respiratory epithelium, metaplasia, squamous		3 (6%)	34 (68%)	49 (98%)
Turbinate, atrophy		50 (100%)	50 (100%)	50 (100%)
Pleura	(1)	(0)	(0)	(0)
Special Senses System				
Eye	(49)	(48)	(49)	(48)
Cataract	1 (2%)	1 (2%)		6 (13%)
Degeneration	1 (2%)			
Phthisis bulbi				1 (2%)
Cornea, epithelium, hyperplasia, focal				1 (2%)
Cornea, inflammation, chronic active		1 (2%)	2 (4%)	10 (21%)
Cornea, ulcer				1 (2%)
Harderian gland	(50)	(49)	(50)	(50)
Hyperplasia	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Hypertrophy				1 (2%)
Inflammation, granulomatous	1 (2%)			
Necrosis	1 (2%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Amyloid deposition				2 (4%)
Infiltration cellular, mononuclear cell	1 (2%)			
Inflammation, suppurative			1 (2%)	
Metaplasia, osseous	1 (2%)	1 (2%)		1 (2%)
Nephropathy	20 (40%)	19 (38%)	19 (38%)	24 (48%)
Thrombosis				1 (2%)
Artery, inflammation, chronic active		1 (2%)		
Capillary, glomerulus, hyperplasia			1 (2%)	1 (2%)
Urinary bladder	(50)	(47)	(48)	(49)
Infiltration cellular, mixed cell		1 (2%)		
Artery, inflammation, chronic active	1 (2%)	1 (2%)		

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1992). Propargyl alcohol was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of propargyl alcohol. The high dose was limited by experimental design to 10,000 µg/plate. All positive trials were repeated under the conditions that elicited the positive response.

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

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female B6C3F1 mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 1,000 normochromatic erythrocytes (NCEs) in each of 10 animals per exposure 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 an exposure group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposed group and the chamber control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month study were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however,

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

RESULTS

Propargyl alcohol (3 to 10,000 µg/plate) was mutagenic in *S. typhimurium* strain TA100 in the absence of liver S9 activation enzymes only; no mutagenicity was observed in TA100 in the presence of liver S9 enzymes (Table E1). In addition, propargyl alcohol was not mutagenic in *S. typhimurium* strain TA1535 without S9 or in TA98 with or without S9. *In vivo*, no significant increases in micronucleated normochromatic erythrocytes were seen in peripheral blood of male B6C3F1 mice exposed to 4, 8, 16, 32, or 64 ppm for 3 months by inhalation (Table E2). All groups of exposed male mice had micronucleated erythrocyte frequencies that were higher than the frequency in the chamber control group, but none of the increases reached statistical significance (P=0.005), and level of response was not related to dose. In the female mice, the trend test was significant (P=0.002), but none of the individual exposure groups had micronucleated erythrocyte frequencies that were significantly increased over the chamber control group, and therefore, the test in female mice was judged to be equivocal. No significant changes in the percentage of PCEs were observed in either male or female mice over the exposure range tested, indicating an absence of treatment-related toxicity to the bone marrow.

TABLE E1
Mutagenicity of Propargyl Alcohol in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+hamster S9 30%	+rat S9 30%
TA100	0	102 ± 2.0	126 ± 12.0	117 ± 8.0	129 ± 12.0	126 ± 8.0
	3				131 ± 2.0	128 ± 8.0
	10				138 ± 16.0	114 ± 8.0
	33				132 ± 2.0	120 ± 10.0
	100	108 ± 10.0			122 ± 9.0	114 ± 1.0
	333	117 ± 3.0	142 ± 2.0	132 ± 7.0	104 ± 7.0	136 ± 8.0
	666			143 ± 6.0		
	1,000	143 ± 3.0	146 ± 14.0	153 ± 11.0	51 ± 14.0[c]	0 ± 0.0[c,d]
	1,666		181 ± 12.0	181 ± 4.0		
	3,333	228 ± 7.0	239 ± 3.0	226 ± 12.0		
	6,666		168 ± 12.0			
	10,000	75 ± 13.0[c]				
Trial summary		Positive	Positive	Positive	Negative	Negative
Positive control[e]		781 ± 17.0	658 ± 95.0	855 ± 13.0	663 ± 31.0	553 ± 52.0
TA1535	0	11 ± 2.0				
	333	10 ± 1.0				
	1,000	8 ± 1.0				
	1,666	8 ± 1.0				
	3,333	10 ± 1.0				
	6,666	5 ± 1.0				
Trial summary		Negative				
Positive control		572 ± 14.0				
TA98	0	16 ± 3.0			23 ± 1.0	24 ± 0.0
	3				27 ± 2.0	18 ± 2.0
	10	17 ± 0.0			34 ± 3.0	22 ± 6.0
	33	14 ± 2.0			44 ± 3.0	30 ± 4.0
	100	19 ± 2.0			43 ± 6.0	29 ± 1.0
	333	14 ± 1.0			43 ± 2.0	25 ± 3.0
	1,000	17 ± 2.0			10 ± 2.0[c]	0 ± 0.0[c,d]
	3,333	11 ± 1.0				
	6,666	11 ± 1.0				
	10,000					
Trial summary		Negative			Equivocal	Negative
Positive control		209 ± 9.0			237 ± 16.0	251 ± 21.0

[a] Study 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] Precipitate seen on plate
[e] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535) and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Propargyl Alcohol by Inhalation for 3 Months[a]

Concentration (ppm)	with Erythrocytes Scored	Number of Mice Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs (%)[b]
Male				
Chamber control	10	3.40 ± 0.54		4.1 ± 0.1
4	10	5.70 ± 0.73	0.0078	4.1 ± 0.2
8	10	5.70 ± 0.72	0.0078	4.0 ± 0.2
16	10	5.40 ± 0.54	0.0163	4.0 ± 0.2
32	10	5.60 ± 0.60	0.0101	4.4 ± 0.2
64	10	4.70 ± 0.72	0.0739	4.2 ± 0.1
		P=0.454[d]		
Female				
Chamber control	10	4.00 ± 0.47		3.6 ± 0.3
4	10	3.00 ± 0.61	0.8844	3.7 ± 0.2
8	10	3.60 ± 0.45	0.6771	4.2 ± 0.2
16	10	3.40 ± 0.31	0.7577	3.6 ± 0.2
32	10	4.60 ± 0.43	0.2584	3.7 ± 0.2
64	10	5.50 ± 0.93	0.0615	3.7 ± 0.2
		P=0.002		

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

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm	64 ppm
Male						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	9	10	10	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 3	45.8 ± 0.4	45.0 ± 0.3	44.5 ± 0.5	45.6 ± 0.6	45.4 ± 0.4	47.0 ± 0.4
Day 23	47.0 ± 0.5	46.9 ± 0.7	47.6 ± 0.3	46.7 ± 0.3	46.6 ± 0.4	47.0 ± 0.4
Week 14	42.8 ± 0.6	42.7 ± 0.4	43.4 ± 0.3	43.7 ± 0.3	43.5 ± 0.5	42.3 ± 0.3
Packed red cell volume (mL/dL)						
Day 3	44.0 ± 0.4	43.0 ± 0.4	42.9 ± 0.5	44.0 ± 0.6	43.5 ± 0.4	45.4 ± 0.5
Day 23	45.4 ± 0.6	44.5 ± 0.7	45.6 ± 0.3	44.8 ± 0.3	44.4 ± 0.5	45.6 ± 0.7
Week 14	42.3 ± 0.5	42.5 ± 0.5	43.5 ± 0.5	43.2 ± 0.3	42.6 ± 0.5	41.5 ± 0.2
Hemoglobin (g/dL)						
Day 3	13.9 ± 0.1	13.7 ± 0.1	13.6 ± 0.2	14.1 ± 0.2	14.0 ± 0.1	14.6 ± 0.1**
Day 23	14.7 ± 0.2	14.6 ± 0.2	14.9 ± 0.1	14.5 ± 0.1	14.6 ± 0.1	14.8 ± 0.2
Week 14	14.8 ± 0.2	14.6 ± 0.1	14.8 ± 0.1	14.6 ± 0.1	14.5 ± 0.1	14.2 ± 0.1**
Erythrocytes (10^6/µL)						
Day 3	6.80 ± 0.08	6.71 ± 0.07	6.71 ± 0.12	6.89 ± 0.09	6.84 ± 0.07	7.19 ± 0.09*
Day 23	7.50 ± 0.11	7.32 ± 0.13	7.64 ± 0.11	7.56 ± 0.09	7.44 ± 0.11	7.67 ± 0.13
Week 14	7.88 ± 0.11	7.96 ± 0.10	8.18 ± 0.08	8.17 ± 0.07	8.17 ± 0.08	8.08 ± 0.05
Reticulocytes (10^6/µL)						
Day 3	0.35 ± 0.04	0.39 ± 0.03	0.41 ± 0.04	0.31 ± 0.04	0.33 ± 0.03	0.32 ± 0.05
Day 23	0.23 ± 0.02	0.26 ± 0.03	0.28 ± 0.02	0.26 ± 0.02	0.25 ± 0.02	0.31 ± 0.02
Week 14	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.20 ± 0.02
Nucleated erythrocytes/100 leukocytes						
Day 3	0.80 ± 0.36	0.90 ± 0.55	0.80 ± 0.33	0.40 ± 0.22	1.00 ± 0.49	0.40 ± 0.16
Day 23	0.60 ± 0.16	0.60 ± 0.31	0.56 ± 0.24	0.30 ± 0.21	0.50 ± 0.17	0.40 ± 0.22
Week 14	0.10 ± 0.10	0.10 ± 0.10	0.40 ± 0.16	0.30 ± 0.15	0.10 ± 0.10	0.10 ± 0.10
Mean cell volume (fL)						
Day 3	64.6 ± 0.4	64.2 ± 0.4	64.0 ± 0.4	64.0 ± 0.2	63.7 ± 0.3	63.1 ± 0.4**
Day 23	60.5 ± 0.4	60.7 ± 0.4	60.0 ± 0.9	59.2 ± 0.4	59.6 ± 0.6	59.5 ± 0.4
Week 14	53.6 ± 0.2	53.4 ± 0.2	53.3 ± 0.2	52.9 ± 0.2*	52.2 ± 0.2**	51.4 ± 0.2**
Mean cell hemoglobin (pg)						
Day 3	20.5 ± 0.2	20.5 ± 0.2	20.4 ± 0.3	20.6 ± 0.1	20.6 ± 0.1	20.4 ± 0.1
Day 23	19.7 ± 0.2	20.1 ± 0.2	19.5 ± 0.3	19.3 ± 0.1	19.7 ± 0.2	19.3 ± 0.2
Week 14	18.8 ± 0.1	18.4 ± 0.1	18.1 ± 0.1**	18.0 ± 0.1**	17.8 ± 0.1**	17.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 3	31.7 ± 0.2	32.1 ± 0.3	31.8 ± 0.3	32.2 ± 0.2	32.3 ± 0.2	32.3 ± 0.2
Day 23	32.5 ± 0.3	33.0 ± 0.2	32.6 ± 0.1	32.6 ± 0.2	33.0 ± 0.2	32.6 ± 0.3
Week 14	35.0 ± 0.2	34.4 ± 0.3	34.0 ± 0.2*	33.9 ± 0.2*	34.3 ± 0.3	34.4 ± 0.2
Platelets (10^3/µL)						
Day 3	757.0 ± 17.7	758.9 ± 17.9	773.2 ± 15.5	796.4 ± 15.6	760.2 ± 24.7	768.1 ± 13.1
Day 23	691.1 ± 17.3	671.1 ± 62.1	744.1 ± 9.4	745.1 ± 14.4	674.1 ± 50.6	741.8 ± 16.9
Week 14	580.7 ± 12.7	587.0 ± 10.4	613.8 ± 9.3	587.6 ± 8.2	592.0 ± 16.7	611.1 ± 10.2
Leukocytes (10^3/µL)						
Day 3	10.10 ± 0.36	9.11 ± 0.49	9.50 ± 0.35	11.16 ± 0.61	9.43 ± 0.53	9.26 ± 0.41
Day 23	3.17 ± 0.20	3.28 ± 0.22	3.22 ± 0.32	3.85 ± 0.72	3.49 ± 0.38	4.05 ± 0.44
Week 14	7.40 ± 0.55	7.17 ± 0.41	6.45 ± 0.39	7.73 ± 0.22	8.03 ± 0.37	7.53 ± 0.46
Segmented neutrophils (10^3/µL)						
Day 3	1.12 ± 0.08	1.12 ± 0.16	1.27 ± 0.13	1.16 ± 0.10	1.02 ± 0.06	1.23 ± 0.14
Day 23	0.43 ± 0.05	0.40 ± 0.05	0.41 ± 0.06	0.43 ± 0.06	0.42 ± 0.06	0.51 ± 0.07
Week 14	1.26 ± 0.16	1.04 ± 0.08	1.11 ± 0.12	1.24 ± 0.15	1.21 ± 0.11	1.22 ± 0.07

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of Propargyl Alcohol

	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm	64 ppm
Male (continued)						
Hematology (continued)						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	9	10	10	10
Week 14	10	10	10	10	10	10
Bands ($10^3/\mu L$)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Lymphocytes ($10^3/\mu L$)						
Day 3	8.87 ± 0.36	7.92 ± 0.41	8.06 ± 0.36	9.90 ± 0.57	8.32 ± 0.55	7.97 ± 0.33
Day 23	2.65 ± 0.20	2.76 ± 0.21	2.73 ± 0.27	3.29 ± 0.62	2.96 ± 0.31	3.43 ± 0.41
Week 14	6.05 ± 0.45	5.95 ± 0.38	5.20 ± 0.35	6.31 ± 0.23	6.59 ± 0.33	6.20 ± 0.46
Monocytes ($10^3/\mu L$)						
Day 3	0.07 ± 0.02	0.05 ± 0.02	0.12 ± 0.04	0.06 ± 0.05	0.07 ± 0.02	0.02 ± 0.01
Day 23	0.09 ± 0.03	0.10 ± 0.03	0.08 ± 0.02	0.12 ± 0.04	0.09 ± 0.03	0.10 ± 0.03
Week 14	0.06 ± 0.02	0.12 ± 0.03	0.07 ± 0.02	0.09 ± 0.03	0.12 ± 0.03	0.07 ± 0.03
Basophils ($10^3/\mu L$)						
Day 3	0.000 ± 0.000	0.000 ± 0.000	0.009 ± 0.009	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.005 ± 0.005
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.006 ± 0.006	0.014 ± 0.009	0.009 ± 0.009	0.000 ± 0.000
Eosinophils ($10^3/\mu L$)						
Day 3	0.03 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.02 ± 0.01	0.05 ± 0.02
Day 23	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Week 14	0.04 ± 0.01	0.07 ± 0.02	0.06 ± 0.02	0.07 ± 0.03	0.09 ± 0.03	0.05 ± 0.03
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	7.9 ± 0.4	6.6 ± 0.3	6.4 ± 0.3	7.2 ± 0.4	10.6 ± 0.6*	16.0 ± 0.4**
Day 23	8.8 ± 0.5	9.1 ± 0.4	9.3 ± 0.3	9.4 ± 0.2	10.3 ± 0.4**	13.7 ± 0.4**
Week 14	13.5 ± 0.4	14.3 ± 0.4	13.3 ± 0.5	14.7 ± 0.4	13.4 ± 0.4	12.2 ± 0.5
Creatinine (mg/dL)						
Day 3	0.79 ± 0.02	0.78 ± 0.01	0.75 ± 0.02	0.73 ± 0.02	0.75 ± 0.02	0.75 ± 0.02
Day 23	0.89 ± 0.02	0.83 ± 0.02	0.82 ± 0.01*	0.85 ± 0.02*	0.81 ± 0.02**	0.79 ± 0.03**
Week 14	0.93 ± 0.02	0.98 ± 0.03	0.98 ± 0.01	0.96 ± 0.02	0.96 ± 0.03	0.91 ± 0.01
Total protein (g/dL)						
Day 3	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.5 ± 0.1
Day 23	6.2 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.4 ± 0.1*
Week 14	6.7 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.8 ± 0.1
Albumin (g/dL)						
Day 3	3.7 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	3.6 ± 0.1	3.7 ± 0.1	3.7 ± 0.0
Day 23	3.8 ± 0.1	3.8 ± 0.0	3.9 ± 0.1	3.8 ± 0.0	3.8 ± 0.0	3.8 ± 0.1
Week 14	3.8 ± 0.1	3.8 ± 0.1	4.0 ± 0.1	3.8 ± 0.1	3.8 ± 0.0	3.9 ± 0.1
Globulin (g/dL)						
Day 3	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.0	1.9 ± 0.0	1.8 ± 0.1	1.8 ± 0.1
Day 23	2.4 ± 0.1	2.4 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.6 ± 0.1
Week 14	2.8 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.9 ± 0.1

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of Propargyl Alcohol

	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm	64 ppm
Male (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Albumin/globulin ratio						
Day 3	2.0 ± 0.1	1.9 ± 0.1	1.8 ± 0.0	1.9 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
Day 23	1.6 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
Week 14	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.1
Alanine aminotransferase (IU/L)						
Day 3	57 ± 2	57 ± 1	55 ± 1	51 ± 1*	53 ± 2*	51 ± 2*
Day 23	43 ± 1	41 ± 1	39 ± 1**	39 ± 1**	37 ± 1**	36 ± 1**
Week 14	87 ± 5	108 ± 9	82 ± 3	102 ± 8	68 ± 4*	69 ± 5*
Alkaline phosphatase (IU/L)						
Day 3	789 ± 21	775 ± 11	759 ± 31	712 ± 18	744 ± 26	758 ± 21
Day 23	514 ± 14	510 ± 14	514 ± 13	512 ± 9	511 ± 11	475 ± 12
Week 14	265 ± 7	275 ± 6	284 ± 7	282 ± 7	273 ± 6	255 ± 3
Creatine kinase (IU/L)						
Day 3	362 ± 28	314 ± 28	314 ± 23[b]	307 ± 23[b]	301 ± 16	358 ± 40
Day 23	267 ± 22	304 ± 30	258 ± 35	267 ± 35	240 ± 17	293 ± 28
Week 14	130 ± 20	208 ± 37	137 ± 12	128 ± 23	168 ± 41	151 ± 19
Sorbitol dehydrogenase (IU/L)						
Day 3	11 ± 0	11 ± 0	11 ± 1	12 ± 1	12 ± 1	14 ± 2
Day 23	11 ± 1	12 ± 0	11 ± 0	11 ± 0	11 ± 0	12 ± 0
Week 14	23 ± 1	29 ± 4	25 ± 2	26 ± 2	21 ± 1	22 ± 1
Cholinesterase (IU/L)						
Day 3	1,094.0 ± 24.0	1,045.0 ± 16.0	1,065.0 ± 13.0	1,046.0 ± 18.0	1,076.0 ± 19.0	1,142.0 ± 29.0
Day 23	991.2 ± 34.4	964.8 ± 46.1	948.5 ± 34.3	934.2 ± 25.0	878.5 ± 29.7**	839.4 ± 27.0**
Week 14	1,071.0 ± 26.2	1,085.5 ± 67.4	1,045.8 ± 30.6	1,100.5 ± 41.2	897.7 ± 25.5**	777.8 ± 25.3**
Bile acids (µmol/L)						
Day 3	29.1 ± 0.7	27.3 ± 0.6	27.5 ± 2.8	22.2 ± 1.5**	25.8 ± 0.6	36.0 ± 3.2
Day 23	27.4 ± 1.2	27.7 ± 1.2	25.3 ± 1.2	25.5 ± 0.6	26.5 ± 1.0	27.0 ± 1.9
Week 14	24.7 ± 0.7	34.3 ± 4.5	27.4 ± 1.4	26.5 ± 0.7	23.8 ± 1.0	24.2 ± 0.8
Hemolysis						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Urinalysis (Week 12)						
n	10	10	10	10	10	10
Creatinine (mg/dL)	42.30 ± 6.94	50.10 ± 5.92	44.10 ± 7.15	47.10 ± 6.61	42.00 ± 6.56	39.00 ± 7.72
Glucose (mg/dL)	5 ± 1	7 ± 1	6 ± 1	7 ± 1	6 ± 1	6 ± 1
Glucose/creatinine ratio	0.129 ± 0.007	0.139 ± 0.005	0.142 ± 0.006	0.139 ± 0.004	0.135 ± 0.006	0.139 ± 0.007
Protein (mg/dL)	28 ± 4	33 ± 4	34 ± 5	34 ± 5	32 ± 4	33 ± 6
Protein/creatinine ratio	0.679 ± 0.040	0.665 ± 0.022	0.776 ± 0.030	0.715 ± 0.016	0.776 ± 0.033	0.843 ± 0.041**
Alkaline phosphatase (IU/L)	153 ± 25	161 ± 19	158 ± 16	169 ± 21	150 ± 18	134 ± 23
Alkaline phosphatase/ creatinine ratio	3.636 ± 0.196	3.271 ± 0.209	3.816 ± 0.281	3.670 ± 0.120	3.772 ± 0.308	3.497 ± 0.206
Aspartate aminotransferase (IU/L)	2 ± 1	3 ± 1	3 ± 1	2 ± 1	1 ± 0	1 ± 1
Aspartate aminotransferase/ creatinine ratio	0.041 ± 0.010	0.053 ± 0.013	0.061 ± 0.009	0.042 ± 0.006	0.030 ± 0.007	0.024 ± 0.007

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of Propargyl Alcohol

	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm	64 ppm
Male (continued)						
Urinalysis (Week 12) (continued)						
n	10	10	10	10	10	10
Lactate dehydrogenase (IU/L)	26 ± 4	30 ± 3	28 ± 4	29 ± 4	24 ± 3	24 ± 4
Lactate dehydrogenase/ creatinine ratio	0.629 ± 0.030	0.608 ± 0.018	0.653 ± 0.028	0.617 ± 0.028	0.601 ± 0.035	0.633 ± 0.033
γ-Glutamyltransferase (IU/L)	1,006 ± 138	1,120 ± 122	1,088 ± 189	1,131 ± 128	906 ± 90	781 ± 148
γ-Glutamyltransferase/ creatinine ratio	24.44 ± 1.01	22.80 ± 1.16	24.62 ± 0.82	24.84 ± 1.07	23.02 ± 1.40	20.11 ± 0.46*
N-acetyl-β-D-glucosaminidase (IU/L)	7 ± 1	8 ± 1	8 ± 1	8 ± 1	7 ± 1	7 ± 1
N-acetyl-β-D-glucosaminidase/ creatinine ratio	0.171 ± 0.007	0.165 ± 0.009	0.175 ± 0.008	0.164 ± 0.005	0.179 ± 0.007	0.180 ± 0.007
Volume (mL/16 hr)	19.5 ± 2.6	16.5 ± 2.1	17.7 ± 2.3	16.3 ± 2.2	19.2 ± 2.5	24.3 ± 3.2
Specific gravity	1.011 ± 0.002	1.013 ± 0.002	1.012 ± 0.002	1.013 ± 0.002	1.012 ± 0.002	1.012 ± 0.002
Female						
Hematology						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	9	9
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 3	46.6 ± 0.7	46.2 ± 0.5	46.1 ± 0.5	46.1 ± 0.6	47.1 ± 1.1	46.9 ± 0.3
Day 23	48.9 ± 0.4	48.5 ± 0.4	49.0 ± 0.4	48.2 ± 0.5	48.5 ± 0.2	48.9 ± 0.6
Week 14	43.1 ± 0.5	43.0 ± 0.5	43.0 ± 0.4	42.4 ± 0.3	43.7 ± 0.5	42.1 ± 0.3
Packed red cell volume (mL/dL)						
Day 3	45.6 ± 0.8	44.9 ± 0.7	44.8 ± 0.6	45.4 ± 0.7	46.5 ± 1.6	47.0 ± 0.7
Day 23	47.9 ± 0.7	47.6 ± 0.4	47.8 ± 0.5	47.3 ± 0.6	47.6 ± 0.4	47.9 ± 0.6
Week 14	42.7 ± 0.2	42.4 ± 0.4	42.0 ± 0.5	41.9 ± 0.6	42.4 ± 0.5	41.1 ± 0.3*
Hemoglobin (g/dL)						
Day 3	14.8 ± 0.2	14.7 ± 0.2	14.6 ± 0.2	14.8 ± 0.2	15.0 ± 0.4	15.3 ± 0.1
Day 23	15.7 ± 0.1	15.5 ± 0.2	15.7 ± 0.1	15.4 ± 0.2	15.5 ± 0.1	15.9 ± 0.2
Week 14	14.8 ± 0.1	14.7 ± 0.1	14.7 ± 0.1	14.6 ± 0.1	14.9 ± 0.1	14.4 ± 0.1
Erythrocytes (10^6/μL)						
Day 3	7.06 ± 0.12	6.97 ± 0.11	6.93 ± 0.09	7.10 ± 0.10	7.29 ± 0.25	7.36 ± 0.12
Day 23	7.82 ± 0.11	7.73 ± 0.06	7.79 ± 0.10	7.69 ± 0.12	7.66 ± 0.10	7.83 ± 0.11
Week 14	7.44 ± 0.05	7.39 ± 0.06	7.38 ± 0.08	7.38 ± 0.10	7.49 ± 0.08	7.27 ± 0.06
Reticulocytes (10^6/μL)						
Day 3	0.28 ± 0.03	0.34 ± 0.04	0.33 ± 0.03	0.33 ± 0.03	0.28 ± 0.04	0.31 ± 0.04
Day 23	0.12 ± 0.02	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.14 ± 0.02	0.17 ± 0.01**
Week 14	0.14 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
Nucleated erythrocytes/100 leukocytes						
Day 3	0.30 ± 0.15	0.10 ± 0.10	0.20 ± 0.13	0.00 ± 0.00	0.40 ± 0.16	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.30 ± 0.15	0.90 ± 0.31	0.40 ± 0.22	0.50 ± 0.22	0.20 ± 0.13	0.30 ± 0.15

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of Propargyl Alcohol

	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm	64 ppm
Female (continued)						
Hematology (continued)						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	9	9
Week 14	10	10	10	10	10	10
Mean cell volume (fL)						
Day 3	64.7 ± 0.3	64.4 ± 0.3	64.6 ± 0.2	63.9 ± 0.3	63.9 ± 0.2*	63.7 ± 0.3*
Day 23	61.1 ± 0.4	61.7 ± 0.7	61.5 ± 0.4	61.3 ± 0.5	62.1 ± 0.5	61.2 ± 0.5
Week 14	57.5 ± 0.2	57.3 ± 0.2	56.9 ± 0.3	56.8 ± 0.3*	56.6 ± 0.2**	56.5 ± 0.2**
Mean cell hemoglobin (pg)						
Day 3	21.0 ± 0.2	21.0 ± 0.2	21.0 ± 0.2	20.8 ± 0.2	20.6 ± 0.2	20.8 ± 0.3
Day 23	20.1 ± 0.2	20.0 ± 0.2	20.1 ± 0.2	20.0 ± 0.2	20.3 ± 0.2	20.3 ± 0.2
Week 14	19.9 ± 0.1	20.0 ± 0.1	20.0 ± 0.2	19.9 ± 0.2	19.9 ± 0.1	19.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	32.4 ± 0.3	32.6 ± 0.2	32.5 ± 0.2	32.5 ± 0.3	32.3 ± 0.3	32.6 ± 0.4
Day 23	32.8 ± 0.3	32.5 ± 0.1	32.7 ± 0.3	32.5 ± 0.3	32.6 ± 0.2	33.1 ± 0.1
Week 14	34.7 ± 0.2	34.8 ± 0.2	35.2 ± 0.3	35.0 ± 0.4	35.1 ± 0.2	35.1 ± 0.2
Platelets (10^3/µL)						
Day 3	669.9 ± 19.3	657.1 ± 21.0	695.3 ± 17.6	664.7 ± 18.5	650.2 ± 30.0	666.4 ± 26.2
Day 23	665.2 ± 15.9	682.3 ± 16.9	688.8 ± 15.8	673.5 ± 12.7	669.8 ± 13.4	676.7 ± 16.7
Week 14	601.7 ± 7.0	611.5 ± 13.5	612.3 ± 8.5	600.2 ± 10.3	639.7 ± 11.7	624.7 ± 8.8
Leukocytes (10^3/µL)						
Day 3	11.94 ± 0.31	11.51 ± 0.39	10.60 ± 0.49	11.40 ± 0.29	10.23 ± 0.53	10.17 ± 0.64
Day 23	7.05 ± 0.54	7.45 ± 0.55	6.90 ± 0.52	6.96 ± 0.35	7.63 ± 0.47	8.33 ± 0.75
Week 14	6.03 ± 0.41	5.47 ± 0.20	5.99 ± 0.57	6.20 ± 0.40	6.32 ± 0.34	6.44 ± 0.28
Segmented neutrophils (10^3/µL)						
Day 3	1.05 ± 0.09	1.13 ± 0.07	1.03 ± 0.14	0.79 ± 0.06	0.93 ± 0.08	0.74 ± 0.09
Day 23	0.86 ± 0.10	0.83 ± 0.11	0.81 ± 0.12	0.76 ± 0.14	0.70 ± 0.06	0.68 ± 0.09
Week 14	0.94 ± 0.09	1.00 ± 0.11	1.01 ± 0.15	0.82 ± 0.07	0.82 ± 0.08	0.87 ± 0.10
Bands (10^3/µL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)						
Day 3	10.39 ± 0.32	10.06 ± 0.37	9.21 ± 0.46	10.19 ± 0.33	8.95 ± 0.52	9.14 ± 0.62
Day 23	6.08 ± 0.52	6.47 ± 0.49	5.91 ± 0.40	6.08 ± 0.44	6.83 ± 0.41	7.42 ± 0.72
Week 14	4.97 ± 0.35	4.37 ± 0.18	4.90 ± 0.43	5.28 ± 0.36	5.44 ± 0.36	5.48 ± 0.28
Monocytes (10^3/µL)						
Day 3	0.45 ± 0.05	0.28 ± 0.06	0.34 ± 0.07	0.36 ± 0.07	0.32 ± 0.05	0.24 ± 0.07
Day 23	0.07 ± 0.03	0.08 ± 0.03	0.08 ± 0.02	0.09 ± 0.06	0.08 ± 0.04	0.19 ± 0.04
Week 14	0.05 ± 0.03	0.06 ± 0.01	0.04 ± 0.02	0.08 ± 0.02	0.01 ± 0.01	0.03 ± 0.02
Basophils (10^3/µL)						
Day 3	0.000 ± 0.000	0.000 ± 0.000	0.010 ± 0.010	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.011 ± 0.008	0.000 ± 0.000	0.008 ± 0.008	0.007 ± 0.007	0.000 ± 0.000	0.009 ± 0.009
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)						
Day 3	0.04 ± 0.02	0.05 ± 0.04	0.00 ± 0.00	0.06 ± 0.03	0.02 ± 0.01	0.05 ± 0.03
Day 23	0.04 ± 0.02	0.07 ± 0.02	0.09 ± 0.06	0.02 ± 0.02	0.02 ± 0.01	0.04 ± 0.02
Week 14	0.07 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.04 ± 0.01	0.07 ± 0.02

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of Propargyl Alcohol

	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm	64 ppm
Female (continued)						
Clinical Chemistry						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	9	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	7.8 ± 0.4	8.1 ± 0.4	7.7 ± 0.4	8.8 ± 0.5	10.8 ± 1.0**	15.3 ± 0.6**
Day 23	11.4 ± 0.5	10.8 ± 0.7	12.1 ± 0.4	12.1 ± 0.4	12.2 ± 0.6	13.1 ± 0.5*
Week 14	14.6 ± 0.4	14.2 ± 0.6	14.5 ± 0.4	15.3 ± 0.6	14.3 ± 0.7	13.7 ± 0.4
Creatinine (mg/dL)						
Day 3	0.78 ± 0.02	0.78 ± 0.01	0.77 ± 0.02	0.73 ± 0.02	0.73 ± 0.02	0.75 ± 0.02
Day 23	0.86 ± 0.02	0.84 ± 0.02	0.86 ± 0.02	0.83 ± 0.02	0.82 ± 0.02	0.83 ± 0.02
Week 14	0.99 ± 0.02	0.96 ± 0.02	0.94 ± 0.02	0.93 ± 0.02	0.94 ± 0.02	0.90 ± 0.02**
Total protein (g/dL)						
Day 3	5.6 ± 0.1	5.5 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.4 ± 0.1	5.4 ± 0.0
Day 23	6.2 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.1 ± 0.1
Week 14	7.2 ± 0.1	7.2 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	7.0 ± 0.1	6.7 ± 0.1**
Albumin (g/dL)						
Day 3	3.6 ± 0.1	3.5 ± 0.1	3.7 ± 0.1	3.7 ± 0.0	3.5 ± 0.1	3.6 ± 0.1
Day 23	4.0 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	3.9 ± 0.0	4.0 ± 0.1	4.0 ± 0.1
Week 14	4.5 ± 0.1	4.5 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.1 ± 0.1**
Globulin (g/dL)						
Day 3	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.0	1.8 ± 0.0
Day 23	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.1
Week 14	2.7 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.6 ± 0.0
Albumin/globulin ratio						
Day 3	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	2.0 ± 0.1
Day 23	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.2	1.9 ± 0.1
Week 14	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.0	1.6 ± 0.1
Alanine aminotransferase (IU/L)						
Day 3	44 ± 1	43 ± 1	42 ± 1	41 ± 1	44 ± 1	43 ± 2
Day 23	34 ± 1	35 ± 1	34 ± 1	32 ± 1	32 ± 1	30 ± 1*
Week 14	60 ± 5	60 ± 3	56 ± 4	68 ± 3	52 ± 4	34 ± 1**
Alkaline phosphatase (IU/L)						
Day 3	613 ± 20	608 ± 16	590 ± 22	543 ± 16*	551 ± 16*	562 ± 23*
Day 23	346 ± 10	386 ± 8*	352 ± 8	338 ± 9	352 ± 8	327 ± 6
Week 14	241 ± 8	237 ± 12	243 ± 9	224 ± 8	246 ± 5	245 ± 7
Creatine kinase (IU/L)						
Day 3	393 ± 56	300 ± 22	328 ± 26	291 ± 31	320 ± 41	386 ± 60
Day 23	237 ± 30	241 ± 34	282 ± 65	183 ± 23	412 ± 110	258 ± 43
Week 14	143 ± 19	145 ± 24	147 ± 14	167 ± 13	129 ± 12	174 ± 26
Sorbitol dehydrogenase (IU/L)						
Day 3	11 ± 1	10 ± 0	11 ± 1	10 ± 0	10 ± 1	11 ± 1
Day 23	12 ± 1	12 ± 0	12 ± 0	12 ± 0	11 ± 1	14 ± 1
Week 14	19 ± 1	17 ± 1	17 ± 1	19 ± 1	18 ± 1	13 ± 1**
Cholinesterase (IU/L)						
Day 3	1,999.0 ± 171.0	1,728.0 ± 85.0	1,890.0 ± 107.0	1,801.0 ± 90.0	1,504.0 ± 56.0*	1,614.0 ± 52.0
Day 23	4,407.4 ± 146.7	3,863.2 ± 194.8	4,298.4 ± 177.0	3,843.2 ± 115.9*	3,125.7 ± 155.1**	2,558.3 ± 86.6**
Week 14	7,845.9 ± 153.0	7,612.5 ± 181.6	7,001.5 ± 309.6*	6,921.3 ± 185.4**	6,341.3 ± 189.1**	3,900.1 ± 186.5**

TABLE F1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Inhalation Study of Propargyl Alcohol

	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm	64 ppm
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	9	9
Week 14	10	10	10	10	10	10
Bile acids (μmol/L)						
Day 3	24.5 ± 0.9	24.6 ± 0.6	25.4 ± 1.6	25.3 ± 1.0	23.8 ± 0.8	28.2 ± 1.8
Day 23	22.1 ± 1.5	23.1 ± 2.7	21.6 ± 0.9	21.3 ± 1.5	21.3 ± 1.7	22.0 ± 1.7
Week 14	24.5 ± 1.2	25.8 ± 2.7	26.7 ± 2.1	25.9 ± 2.3	21.1 ± 1.0	23.7 ± 2.0
Hemolysis						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Urinalysis (Week 12)						
n	10	10	10	10	10	10
Creatinine (mg/dL)	35.90 ± 2.55	22.30 ± 2.11**	35.00 ± 4.94*	19.90 ± 2.13**	17.90 ± 2.22**	22.60 ± 2.60**
Glucose (mg/dL)	4 ± 0	2 ± 0*	4 ± 1	2 ± 0*	2 ± 0**	3 ± 1
Glucose/creatinine ratio	0.117 ± 0.006	0.093 ± 0.010	0.128 ± 0.005	0.108 ± 0.015	0.121 ± 0.015	0.113 ± 0.015
Protein (mg/dL)	3 ± 0	2 ± 0	3 ± 0	1 ± 0**	2 ± 0	3 ± 0
Protein/creatinine ratio	0.096 ± 0.006	0.111 ± 0.007	0.097 ± 0.008	0.071 ± 0.013	0.111 ± 0.012	0.109 ± 0.007
Alkaline phosphatase (IU/L)	85 ± 6	62 ± 6	102 ± 21	58 ± 9	64 ± 7	99 ± 15
Alkaline phosphatase/ creatinine ratio	2.404 ± 0.094	2.827 ± 0.214	2.884 ± 0.297	2.817 ± 0.179	3.607 ± 0.194**	4.323 ± 0.305**
Aspartate aminotransferase (IU/L)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1
Aspartate aminotransferase/ creatinine ratio	0.000 ± 0.000	0.004 ± 0.004	0.002 ± 0.002	0.000 ± 0.000	0.000 ± 0.000	0.033 ± 0.030
Lactate dehydrogenase (IU/L)	15 ± 1	11 ± 1	19 ± 2	12 ± 1	10 ± 1	12 ± 2
Lactate dehydrogenase/ creatinine ratio	0.432 ± 0.020	0.474 ± 0.034	0.564 ± 0.037**	0.606 ± 0.043**	0.592 ± 0.046**	0.517 ± 0.055**
γ-Glutamyltransferase (IU/L)	415 ± 43	272 ± 34	456 ± 101	272 ± 41	275 ± 29	313 ± 50
γ-Glutamyltransferase/ creatinine ratio	11.49 ± 0.770	12.07 ± 0.750	12.41 ± 1.240	13.42 ± 1.010	15.72 ± 0.910**	13.40 ± 0.770*
N-acetyl-β-D-glucosaminidase (IU/L)	5 ± 0	3 ± 0*	5 ± 1	3 ± 0*	3 ± 0**	4 ± 0
N-acetyl-β-D-glucosaminidase/ creatinine ratio	0.145 ± 0.005	0.145 ± 0.006	0.146 ± 0.004	0.145 ± 0.008	0.155 ± 0.005	0.174 ± 0.007**
Volume (mL/16 hr)	10.6 ± 1.0	18.5 ± 2.8	11.8 ± 1.7	20.8 ± 2.7**	23.7 ± 2.3	20.1 ± 3.2**
Specific gravity	1.013 ± 0.001	1.008 ± 0.001	1.013 ± 0.002	1.007 ± 0.001**	1.006 ± 0.001**	1.008 ± 0.001**

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

TABLE F2
Hematology Data for Mice in the 3-Month Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm	64 ppm
n	10	10	10	10	10	10
Male						
Hematocrit (%)	49.5 ± 0.3	48.2 ± 0.4	48.8 ± 0.4	48.8 ± 0.4	47.5 ± 0.4**	47.0 ± 0.3**
Packed red cell volume (mL/dL)	49.9 ± 0.3	48.7 ± 0.4	49.0 ± 0.4	48.7 ± 0.4*	47.8 ± 0.3**	47.0 ± 0.4**
Hemoglobin (g/dL)	15.7 ± 0.1	15.5 ± 0.1	15.5 ± 0.1	15.4 ± 0.1	15.1 ± 0.1**	15.0 ± 0.1**
Erythrocytes (10^6/µL)	9.86 ± 0.06	9.73 ± 0.07	9.82 ± 0.08	9.72 ± 0.08	9.56 ± 0.08**	9.44 ± 0.06**
Reticulocytes (10^6/µL)	0.19 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.19 ± 0.13	0.16 ± 0.02	0.18 ± 0.01
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Howell-Jolly bodies (% RBC)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Mean cell volume (fL)	50.6 ± 0.2	50.1 ± 0.1	49.8 ± 0.2*	50.1 ± 0.1	50.0 ± 0.2	49.9 ± 0.3
Mean cell hemoglobin (pg)	16.0 ± 0.1	15.9 ± 0.1	15.8 ± 0.1	15.9 ± 0.1	15.8 ± 0.1	15.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.5 ± 0.2	31.7 ± 0.1	31.6 ± 0.1	31.6 ± 0.1	31.6 ± 0.1	31.8 ± 0.2
Platelets (10^3/µL)	752.5 ± 17.7	743.2 ± 8.3	752.7 ± 9.6	740.5 ± 20.7	743.9 ± 14.2	727.1 ± 15.3
Leukocytes (10^3/µL)	3.00 ± 0.37	2.57 ± 0.36	2.95 ± 0.36	3.32 ± 0.32	3.59 ± 0.27	3.73 ± 0.38
Segmented neutrophils (10^3/µL)	0.40 ± 0.06	0.38 ± 0.09	0.43 ± 0.09	0.33 ± 0.05	0.41 ± 0.07	0.45 ± 0.10
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	2.57 ± 0.31	2.15 ± 0.28	2.48 ± 0.29	2.94 ± 0.28	3.10 ± 0.21	3.22 ± 0.29
Monocytes (10^3/µL)	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Female						
Hematocrit (%)	49.7 ± 0.3	50.7 ± 0.3	50.3 ± 0.3	50.0 ± 0.5	49.8 ± 0.2	48.7 ± 0.5
Packed red cell volume (mL/dL)	49.6 ± 0.4	50.2 ± 0.4	50.2 ± 0.3	49.7 ± 0.4	49.4 ± 0.2	48.7 ± 0.3
Hemoglobin (g/dL)	15.8 ± 0.1	16.2 ± 0.1	15.9 ± 0.1	15.9 ± 0.1	15.9 ± 0.1	15.5 ± 0.1
Erythrocytes (10^6/µL)	9.65 ± 0.07	9.75 ± 0.08	9.76 ± 0.05	9.74 ± 0.09	9.66 ± 0.05	9.53 ± 0.09
Reticulocytes (10^6/µL)	0.21 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Howell-Jolly bodies (% RBC)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Mean cell volume (fL)	51.5 ± 0.2	51.6 ± 0.2	51.3 ± 0.2	51.0 ± 0.2	51.2 ± 0.2	51.1 ± 0.2
Mean cell hemoglobin (pg)	16.4 ± 0.1	16.6 ± 0.1	16.3 ± 0.1	16.4 ± 0.2	16.5 ± 0.1	16.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.9 ± 0.2	32.3 ± 0.2	31.7 ± 0.2	32.1 ± 0.3	32.1 ± 0.2	31.8 ± 0.1
Platelets (10^3/µL)	688.0 ± 14.1	697.2 ± 19.4	697.0 ± 15.2	663.7 ± 15.0	660.3 ± 21.8	684.1 ± 16.1
Leukocytes (10^3/µL)	3.55 ± 0.35	3.34 ± 0.38	3.24 ± 0.30	3.58 ± 0.35	3.96 ± 0.20	4.34 ± 0.52
Segmented neutrophils (10^3/µL)	0.37 ± 0.08	0.40 ± 0.10	0.40 ± 0.08	0.37 ± 0.05	0.41 ± 0.07	0.60 ± 0.16
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	3.05 ± 0.27	2.83 ± 0.29	2.70 ± 0.22	3.12 ± 0.30	3.46 ± 0.17	3.59 ± 0.40
Monocytes (10^3/µL)	0.06 ± 0.02	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.07 ± 0.02
Basophils (10^3/µL)	0.000 ± 0.000	0.005 ± 0.005	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.06 ± 0.02	0.06 ± 0.01	0.07 ± 0.03	0.04 ± 0.01	0.03 ± 0.01	0.08 ± 0.03

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

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

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Inhalation Study of Propargyl Alcohol[a]

ppm	Chamber Control	31.3 ppm	62.5 ppm	125 ppm	250 ppm	500
Male						
n	5	5	5	0	0	0
Necropsy body wt	161 ± 4	151 ± 5	144 ± 4**			
Heart						
Absolute	0.600 ± 0.018	0.568 ± 0.018	0.548 ± 0.012			
Relative	3.714 ± 0.040	3.759 ± 0.060	3.799 ± 0.058			
R. Kidney						
Absolute	0.594 ± 0.029	0.634 ± 0.028	0.646 ± 0.020			
Relative	3.672 ± 0.116	4.189 ± 0.083**	4.476 ± 0.084**			
Liver						
Absolute	7.132 ± 0.255	6.816 ± 0.376	7.078 ± 0.191			
Relative	44.129 ± 0.759	44.947 ± 0.953	49.061 ± 0.885**			
Lung						
Absolute	1.060 ± 0.066	1.070 ± 0.043	0.962 ± 0.021			
Relative	6.554 ± 0.329	7.109 ± 0.375	6.670 ± 0.088			
R. Testis						
Absolute	0.973 ± 0.019	0.922 ± 0.049	0.904 ± 0.024			
Relative	6.029 ± 0.044	6.086 ± 0.193	6.268 ± 0.160			
Thymus						
Absolute	0.428 ± 0.023	0.446 ± 0.015	0.374 ± 0.025			
Relative	2.651 ± 0.146	2.954 ± 0.097	2.582 ± 0.114			
Female						
n	5	5	5	4	0	0
Necropsy body wt	121 ± 1	115 ± 2	116 ± 3	111 ± 4**		
Heart						
Absolute	0.490 ± 0.005	0.456 ± 0.004*	0.458 ± 0.015	0.488 ± 0.009		
Relative	4.067 ± 0.065	3.961 ± 0.059	3.951 ± 0.060	4.409 ± 0.088**		
R. Kidney						
Absolute	0.490 ± 0.016	0.520 ± 0.009	0.544 ± 0.012**	0.578 ± 0.014**		
Relative	4.063 ± 0.114	4.513 ± 0.034**	4.698 ± 0.054**	5.219 ± 0.090**		
Liver						
Absolute	4.794 ± 0.145	4.796 ± 0.066	5.140 ± 0.115	6.488 ± 0.297**		
Relative	39.757 ± 1.038	41.644 ± 0.523	44.380 ± 0.400**	58.489 ± 0.686**		
Lung						
Absolute	0.842 ± 0.043	0.840 ± 0.023	0.854 ± 0.025	0.885 ± 0.048		
Relative	6.984 ± 0.346	7.297 ± 0.225	7.394 ± 0.310	8.031 ± 0.608		
Thymus						
Absolute	0.324 ± 0.013	0.349 ± 0.013	0.350 ± 0.009	0.323 ± 0.020		
Relative	2.690 ± 0.113	3.035 ± 0.143	3.028 ± 0.073	2.907 ± 0.132		

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

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Inhalation Study of Propargyl Alcohol[a]

ppm	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm 64
n	10	10	10	10	10
Male					
Necropsy body wt	335 ± 4	354 ± 7	333 ± 9	337 ± 9	332 ± 3	328 ± 7
Heart						
Absolute	0.932 ± 0.015	0.994 ± 0.020	0.945 ± 0.017	0.973 ± 0.024	0.964 ± 0.010	0.980 ± 0.019
Relative	2.786 ± 0.035	2.810 ± 0.029	2.845 ± 0.050	2.894 ± 0.026*	2.904 ± 0.023*	2.994 ± 0.041**
R. Kidney						
Absolute	1.002 ± 0.022	1.075 ± 0.026	1.019 ± 0.025	1.050 ± 0.033	1.049 ± 0.019	1.052 ± 0.018
Relative	2.994 ± 0.047	3.038 ± 0.051	3.061 ± 0.029	3.119 ± 0.041	3.159 ± 0.045*	3.217 ± 0.059**
Liver						
Absolute	10.08 ± 0.20	11.21 ± 0.27	10.45 ± 0.42	10.68 ± 0.42	11.00 ± 0.21	11.48 ± 0.30*
Relative	30.114 ± 0.366	31.658 ± 0.343*	31.258 ± 0.525	31.656 ± 0.510*	33.145 ± 0.582**	35.013 ± 0.447**
Lung						
Absolute	1.517 ± 0.059	1.628 ± 0.041	1.550 ± 0.040	1.590 ± 0.065	1.555 ± 0.030	1.646 ± 0.045
Relative	4.529 ± 0.149	4.599 ± 0.060	4.661 ± 0.092	4.734 ± 0.183	4.681 ± 0.068	5.039 ± 0.161**
R. Testis						
Absolute	1.411 ± 0.017	1.443 ± 0.023	1.385 ± 0.018	1.406 ± 0.024	1.391 ± 0.022	1.392 ± 0.03
Relative	4.219 ± 0.057	4.081 ± 0.041	4.177 ± 0.107	4.192 ± 0.070	4.191 ± 0.056	4.249 ± 0.035
Thymus						
Absolute	0.345 ± 0.024	0.399 ± 0.019	0.341 ± 0.023	0.403 ± 0.023	0.362 ± 0.011	0.363 ± 0.012
Relative	1.034 ± 0.073	1.127 ± 0.046	1.027 ± 0.069	1.194 ± 0.052	1.090 ± 0.028	1.108 ± 0.031
Female						
Necropsy body wt	196 ± 5	204 ± 6	190 ± 4	191 ± 4	197 ± 3	190 ± 3
Heart						
Absolute	0.640 ± 0.013	0.629 ± 0.015	0.630 ± 0.012	0.624 ± 0.016	0.636 ± 0.008	0.627 ± 0.010
Relative	3.272 ± 0.048	3.100 ± 0.068	3.315 ± 0.048	3.273 ± 0.048	3.233 ± 0.044	3.303 ± 0.050
R. Kidney						
Absolute	0.649 ± 0.020	0.669 ± 0.018	0.660 ± 0.017	0.656 ± 0.015	0.680 ± 0.015	0.679 ± 0.013
Relative	3.313 ± 0.064	3.294 ± 0.061	3.469 ± 0.048	3.441 ± 0.049	3.454 ± 0.065	3.573 ± 0.039**
Liver						
Absolute	5.878 ± 0.191	5.958 ± 0.220	5.697 ± 0.149	5.861 ± 0.201	6.154 ± 0.121	6.358 ± 0.167
Relative	29.957 ± 0.447	29.260 ± 0.602	29.953 ± 0.522	30.691 ± 0.635	31.268 ± 0.542	33.436 ± 0.565**
Lung						
Absolute	1.257 ± 0.075	1.094 ± 0.025	1.177 ± 0.036	1.189 ± 0.070	1.160 ± 0.022	1.250 ± 0.048
Relative	6.407 ± 0.325	5.416 ± 0.207*	6.195 ± 0.186	6.210 ± 0.277	5.894 ± 0.100	6.593 ± 0.283
Thymus						
Absolute	0.308 ± 0.011	0.324 ± 0.018	0.290 ± 0.015	0.302 ± 0.013	0.310 ± 0.007	0.325 ± 0.013
Relative	1.574 ± 0.058	1.596 ± 0.085	1.526 ± 0.073	1.582 ± 0.050	1.574 ± 0.037	1.712 ± 0.063

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

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Inhalation Study of Propargyl Alcohol[a]

ppm	Chamber Control	31.3 ppm	62.5 ppm	125 ppm	250 ppm	500
n	5	5	5	0	0	0
Male						
Necropsy body wt	26.7 ± 0.8	26.6 ± 0.6	23.6 ± 0.3**			
Heart						
Absolute	0.128 ± 0.004	0.126 ± 0.005	0.110 ± 0.000**			
Relative	4.797 ± 0.129	4.729 ± 0.147	4.667 ± 0.050			
R. Kidney						
Absolute	0.222 ± 0.009	0.254 ± 0.010*	0.226 ± 0.005			
Relative	8.303 ± 0.178	9.526 ± 0.221**	9.593 ± 0.285**			
Liver						
Absolute	1.326 ± 0.047	1.390 ± 0.036	1.324 ± 0.030			
Relative	49.642 ± 1.232	52.187 ± 0.682	56.155 ± 1.178**			
Lung						
Absolute	0.198 ± 0.018	0.186 ± 0.007	0.172 ± 0.005			
Relative	7.414 ± 0.654	6.971 ± 0.123	7.302 ± 0.257			
R. Testis						
Absolute	0.101 ± 0.002	0.106 ± 0.004	0.099 ± 0.001			
Relative	3.802 ± 0.070	3.989 ± 0.124	4.200 ± 0.064*			
Thymus						
Absolute	0.053 ± 0.003	0.049 ± 0.003	0.033 ± 0.003**			
Relative	2.005 ± 0.127	1.843 ± 0.117	1.410 ± 0.139**			
Female						
Necropsy body wt	22.6 ± 0.8	22.6 ± 0.3	20.5 ± 0.5**			
Heart						
Absolute	0.116 ± 0.004	0.118 ± 0.004	0.104 ± 0.004			
Relative	5.134 ± 0.115	5.230 ± 0.142	5.081 ± 0.132			
R. Kidney						
Absolute	0.154 ± 0.004	0.172 ± 0.005*	0.164 ± 0.004			
Relative	6.821 ± 0.141	7.622 ± 0.173**	8.016 ± 0.048**			
Liver						
Absolute	1.126 ± 0.057	1.180 ± 0.027	1.130 ± 0.033			
Relative	49.673 ± 0.805	52.289 ± 0.711*	55.207 ± 0.460**			
Lung						
Absolute	0.188 ± 0.006	0.184 ± 0.004	0.180 ± 0.013			
Relative	8.328 ± 0.235	8.172 ± 0.295	8.790 ± 0.522			
Thymus						
Absolute	0.077 ± 0.004	0.077 ± 0.006	0.059 ± 0.006			
Relative	3.382 ± 0.099	3.417 ± 0.244	2.859 ± 0.217			

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

TABLE G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Inhalation Study of Propargyl Alcohol[a]

ppm	Chamber Control	4 ppm	8 ppm	16 ppm	32 ppm 64
n	10	10	10	10	10	10
Male						
Necropsy body wt	39.8 ± 0.9	38.6 ± 0.4	37.9 ± 0.8	36.4 ± 0.9**	35.3 ± 0.7**	33.6 ± 0.5**
Heart						
Absolute	0.171 ± 0.005	0.169 ± 0.005	0.168 ± 0.004	0.164 ± 0.003	0.152 ± 0.003**	0.156 ± 0.004**
Relative	4.312 ± 0.141	4.374 ± 0.109	4.432 ± 0.094	4.517 ± 0.080	4.313 ± 0.087	4.646 ± 0.097
R. Kidney						
Absolute	0.323 ± 0.005	0.328 ± 0.005	0.335 ± 0.010	0.337 ± 0.007	0.315 ± 0.004	0.298 ± 0.008*
Relative	8.139 ± 0.161	8.495 ± 0.133	8.824 ± 0.149**	9.275 ± 0.155**	8.941 ± 0.147**	8.879 ± 0.229**
Liver						
Absolute	1.631 ± 0.031	1.618 ± 0.030	1.591 ± 0.035	1.561 ± 0.040	1.576 ± 0.054	1.579 ± 0.049
Relative	41.033 ± 0.488	41.906 ± 0.789	41.950 ± 0.553	42.899 ± 0.654	44.599 ± 1.097**	47.054 ± 1.387**
Lung						
Absolute	0.249 ± 0.007	0.233 ± 0.009	0.232 ± 0.008	0.231 ± 0.005	0.228 ± 0.006	0.232 ± 0.012
Relative	6.264 ± 0.140	6.038 ± 0.241	6.101 ± 0.116	6.361 ± 0.155	6.482 ± 0.226	6.898 ± 0.325
R. Testis						
Absolute	0.126 ± 0.002	0.123 ± 0.001	0.124 ± 0.002	0.123 ± 0.002	0.121 ± 0.002	0.124 ± 0.002
Relative	3.169 ± 0.093	3.178 ± 0.027	3.282 ± 0.051	3.390 ± 0.079*	3.433 ± 0.071**	3.683 ± 0.061**
Thymus						
Absolute	0.048 ± 0.004	0.051 ± 0.002	0.046 ± 0.002	0.042 ± 0.002	0.044 ± 0.002	0.042 ± 0.003
Relative	1.193 ± 0.083	1.317 ± 0.056	1.226 ± 0.052	1.160 ± 0.061	1.242 ± 0.062	1.249 ± 0.073
Female						
Necropsy body wt	31.8 ± 0.9	32.0 ± 0.8	31.2 ± 1.1	31.3 ± 1.0	29.6 ± 0.4	28.1 ± 0.4**
Heart						
Absolute	0.136 ± 0.003	0.145 ± 0.002	0.138 ± 0.003	0.146 ± 0.004	0.138 ± 0.002	0.133 ± 0.002
Relative	4.295 ± 0.120	4.547 ± 0.121	4.469 ± 0.165	4.683 ± 0.145*	4.667 ± 0.064*	4.745 ± 0.101*
R. Kidney						
Absolute	0.203 ± 0.005	0.208 ± 0.005	0.206 ± 0.008	0.206 ± 0.004	0.214 ± 0.003	0.207 ± 0.007
Relative	6.416 ± 0.203	6.509 ± 0.146	6.683 ± 0.355	6.620 ± 0.192	7.239 ± 0.076**	7.364 ± 0.171**
Liver						
Absolute	1.440 ± 0.033	1.441 ± 0.043	1.396 ± 0.041	1.426 ± 0.039	1.366 ± 0.026	1.332 ± 0.036
Relative	45.416 ± 0.964	45.000 ± 0.925	44.969 ± 1.065	45.687 ± 1.106	46.177 ± 0.507	47.369 ± 0.594
Lung						
Absolute	0.224 ± 0.008	0.237 ± 0.008	0.230 ± 0.012	0.250 ± 0.011	0.243 ± 0.009	0.235 ± 0.005
Relative	7.093 ± 0.338	7.449 ± 0.345	7.455 ± 0.476	8.059 ± 0.457	8.210 ± 0.268*	8.377 ± 0.178*
Thymus						
Absolute	0.056 ± 0.002	0.062 ± 0.004	0.057 ± 0.002	0.060 ± 0.003	0.057 ± 0.001	0.054 ± 0.004
Relative	1.763 ± 0.076	1.933 ± 0.123	1.844 ± 0.068	1.910 ± 0.090	1.942 ± 0.043	1.923 ± 0.135

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

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	16 ppm	32 ppm	64 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	335 ± 4	337 ± 9	332 ± 3	328 ± 7
L. Cauda epididymis	0.1832 ± 0.0034	0.1718 ± 0.0062	0.1829 ± 0.0070	0.1652 ± 0.0061
L. Epididymis	0.2973 ± 0.0067	0.3005 ± 0.0092	0.2853 ± 0.0086	0.2843 ± 0.0077
L. Testis	1.4738 ± 0.0185	1.4880 ± 0.0615	1.4029 ± 0.0312	1.4114 ± 0.0405
Spermatid measurements				
Spermatid heads (10^6/g testis)	115.5 ± 4.2	109.3 ± 4.4	108.5 ± 3.7	113.0 ± 5.0
Spermatid heads (10^6/testis)	161.0 ± 5.8	153.8 ± 8.5	142.4 ± 6.5	150.8 ± 8.5
Epididymal spermatozoal measurements				
Motility (%)	90.58 ± 0.95	87.73 ± 1.06	86.69 ± 2.32	86.12 ± 1.04
10^6/g cauda epididymal tissue	608.8 ± 25.8	565.3 ± 28.5	593.0 ± 28.1	616.7 ± 19.7
10^6/cauda epididymal tissue	111.1 ± 3.8	100.3 ± 5.0	107.2 ± 3.5	101.2 ± 3.1

[a] Data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test (body and tissue weights and epididymal spermatozoa/cauda measurements) or Dunn's test (spermatid, motility, and epididymal spermatozoa/g cauda measurements).

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	16 ppm	32 ppm	64 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	196 ± 5	191 ± 4	197 ± 3	190 ± 3
Proportion of regular cycling females[b]	6/10	5/10	6/9	7/10
Estrous cycle length (days)	4.85 ± 0.30	4.80 ± 0.23	4.89 ± 0.44[c]	4.90 ± 0.07
Estrous stages (% of cycle)				
Diestrus	29.2	35.8	35.8	34.2
Proestrus	11.7	10.0	7.5	13.3
Estrus	44.2	35.0	39.2	35.8
Metestrus	15.0	19.2	17.5	16.7

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test (body weights), Dunn's test (estrous cycle length), or Fisher's exact test (proportion of regular cycling females). By multivariate analysis of variance, exposed females did not differ significantly from the chamber control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 10 days or unclear in 1 of 10 animals.

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	16 ppm	32 ppm	64 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	39.8 ± 0.9	36.4 ± 0.9**	35.3 ± 0.7**	33.6 ± 0.5**
L. cauda epididymis	0.0176 ± 0.0010	0.0177 ± 0.0013	0.0191 ± 0.0011	0.0151 ± 0.0004
L. epididymis	0.0314 ± 0.0012	0.0335 ± 0.0017	0.0316 ± 0.0008	0.0293 ± 0.0005
L. testis	0.1159 ± 0.0016	0.1198 ± 0.0020	0.1160 ± 0.0011	0.1168 ± 0.0019
Spermatid measurements				
Spermatid heads (10^6/g testis)	183.0 ± 10.8	186.7 ± 7.5	191.1 ± 7.2	196.7 ± 6.8
Spermatid heads (10^6/testis)	19.70 ± 1.23	20.46 ± 0.69	20.28 ± 0.75	20.65 ± 0.79
Epididymal spermatozoal measurements				
Motility (%)	91.26 ± 1.38	89.96 ± 0.57	89.24 ± 1.43	88.29 ± 1.32
10^6/g cauda epididymal tissue	1,004.8 ± 33.7	944.4 ± 85.6	913.3 ± 52.9	1,055.2 ± 49.6
10^6/cauda epididymal tissue	17.69 ± 1.22	15.79 ± 0.44	17.09 ± 0.78	15.87 ± 0.56

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

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Inhalation Study of Propargyl Alcohol[a]

	Chamber Control	16 ppm	32 ppm	64 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	31.8 ± 0.9	31.3 ± 1.0	29.6 ± 0.4*	28.1 ± 0.4**
Proportion of regular cycling females[b]	6/10	6/10	6/10	2/9
Estrous cycle length (days)	3.94 ± 0.08	3.89 ± 0.06	4.36 ± 0.41	4.39 ± 0.17*[c]
Estrous stages[d] (% of cycle)				
Diestrus	23.3	24.2	25.0	26.1
Proestrus	0.0	0.0	0.0	1.7
Estrus	55.0	51.7	52.5	54.6
Metestrus	21.7	24.2	22.5	17.6

* Significantly different ($P \leq 0.05$) from the chamber control group by William's test (body weights) or Dunn's test (estrous cycle length).
** Significantly different ($P \leq 0.01$) from the chamber control group by William's test.
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the chamber control group are not significant by Fisher's exact test for proportion of regular cycling females.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 10 days or unclear in 1 of 10 animals.
[d] Evidence shows that females exposed to 16 ppm differ significantly (Wilk's Criterion, $P \leq 0.05$) from the chamber control females in the relative length of time spent in the estrous stages; these females spent more time in metestrus and less time in estrus than chamber control females.

APPENDIX I
CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

Procurement and Characterization of Propargyl Alcohol

Propargyl alcohol was obtained from International Specialty Products (Texas City, TX) in one lot (04923BI) that was used in the 2-week, 3-month, and 2-year studies. Identity analyses were performed by the analytical chemistry laboratory, Midwest Research Institute (MRI, Kansas City, MO), and the study laboratory, Battelle Toxicology Northwest (Richland, WA). Purity analyses were performed by MRI and the study laboratory; elemental analysis was performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the propargyl alcohol studies are on file at the National Institute of Environmental Health Sciences.

Lot 04923BI, a colorless liquid, was identified as propargyl alcohol by infrared and proton nuclear magnetic resonance spectroscopy. All spectra were consistent with the literature spectra (*Aldrich*, 1985, 1993, 1997) and the structure of propargyl alcohol. Representative infrared and proton nuclear magnetic resonance spectra are presented in Figures I1 and I2.

Elemental analysis was performed by Galbraith Laboratories, and water content was determined by the analytical chemistry laboratory using Karl Fischer titration. MRI determined the purity using gas chromatography (GC) coupled with mass spectroscopy (MS) by system A (Table I1). The study laboratory determined the purity using GC by systems B and C (Table I1), and high-performance liquid chromatography (HPLC) was used to determine the formaldehyde content by a system that included a Hewlett-Packard gas chromatograph, ultraviolet detection at 355 nm, a Phenomenex C_{18} 5 µm column (250 mm × 4.6 mm) (Phenomenex, Torrance, CA), a mobile phase of acetonitrile:water:tetrahydrofuran:2-propanol [A) 30:59:10:1; B) 65:35:0:0; and C) 100:0:0:0], beginning with 100% A to 60:40 A:B in 16 minutes, then 100% C in 0.01 minute, held 7 minutes, then 100% A in 0.01 minute, at a flow rate of 1.2 mL/minute. A solution of possible impurity or degradation products (acetol and 2-butyne-1,4-diol at a relative concentration of 0.1% by weight) was analyzed using GC by system B to show the resolution and selectivity of the method.

Prior to the 2-week studies, elemental analyses for carbon, hydrogen, and oxygen were in agreement with the theoretical values for propargyl alcohol (*Merck*, 1989). Karl Fischer titration indicated 2,011 ppm water. HPLC analysis indicated 0.17% formaldehyde. GC by system A indicated a purity greater than 99.0% with no impurities detected. GC by system B indicated a purity greater than 99.6% relative to an independent standard (Aldrich Chemical Co., Inc.). GC by system C indicated the area percent purity for the major propargyl alcohol peak was 99.9%, and no impurity peaks with an area percent greater than 0.1% relative to the major peak area were detected. The overall purity of lot 04923BI was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored at 2° to 8° C in the original shipping containers (55-gallon metal drums). Stability of the bulk chemical was monitored by the study laboratory using GC by systems B and C within 30 days before the start and within 30 days after the last animal sacrifice of the 3-month studies, within 6 weeks before the start of the 2-year studies, at intervals of 24 ± 2 weeks during the studies, and within 30 days after the last animal sacrifice. No degradation of the bulk chemical was detected.

Vapor Generation and Exposure System

A diagram of the vapor generation and delivery system used in the studies is shown in Figure I3. Propargyl alcohol was pumped onto glass beads in a heated glass column where it was vaporized. Heated nitrogen flowed through the column and carried the vapor to a short vapor distribution manifold, where concentration was controlled by the chemical pump and nitrogen flow rates. Pressure in the distribution manifold was fixed to ensure constant flows through the manifold and into the chambers.

Individual Teflon® delivery lines carried the vapor from the manifold to three-way exposure valves at the chamber inlets. The exposure valves diverted vapor delivery to the exposure chamber exhaust until the generation system stabilized and exposure could proceed. The flow rate to each chamber was controlled by a metering valve at the manifold. To initiate exposure, the chamber exposure valves were rotated to allow the vapor to flow to each exposure chamber inlet duct where it was diluted with conditioned chamber air to achieve the desired exposure concentration.

The study laboratory designed the inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A small-particle detector (Model 3022A; TSI, Inc., St. Paul, MN) was used with and without animals present in the exposure chambers to ensure that propargyl alcohol vapor, and not aerosol, was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm^3) were detected.

Vapor Concentration Monitoring

Summaries of the chamber vapor concentrations are given in Tables I2 through I4. Chamber and room concentrations of propargyl alcohol were monitored by an on-line gas chromatograph (system D, Table I1). Samples were drawn from each exposure chamber approximately every 20 (2-week and 3-month studies) or 24 (2-year studies) minutes during each 6-hour exposure period using Hasteloy-C stream-select and gas-sampling valves (VALCO Instruments Co., Houston, TX) in a separate, heated valve oven. The sample lines composing each sample loop were made from Teflon® tubing and were connected to the exposure chamber relative humidity sampling lines at a location close to the gas chromatograph. A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum regulator and the gas chromatograph allowed digital measurement of sample flow.

The on-line gas chromatograph was checked throughout the day for instrument drift against an on-line standard of propargyl alcohol in nitrogen supplied by a standard generator (Kin-Tek; Precision Calibration Systems, La Marque, TX). The on-line gas chromatograph was calibrated prior to the start of each study, three times during the 2-week studies, and monthly during the 3-month and 2-year studies by a comparison of chamber concentration data to data from grab samples that were collected with adsorbent gas sampling tubes containing silica gel (ORBO-52; Supelco, Bellefonte, PA), extracted with methanol containing 4-methyl-2-pentanol as an internal standard, and analyzed using an off-line GC (system E, Table I1). The volumes of gas were sampled at a constant flow rate ensured by a calibrated critical orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standards of propargyl alcohol and the internal standard (4-methyl-2-pentanol) in methanol.

Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 12.5 minutes. For rats and mice in the 2-week studies, T_{90} values ranged from 7 to 12 minutes with animals present; T_{10} values ranged from 9 to 16 minutes with animals present. For rats and mice in the 3-month studies, T_{90} values were 9 minutes without animals present and from 11 to 13 minutes with animals present; T_{10} values ranged from 9 to 10 minutes without animals present and 14 minutes with animals present. For rats and mice in the 2-year studies, T_{90} values ranged from 9 to 11 minutes without animals present and from 13 to 18 minutes with animals present; T_{10} values were 9 minutes without animals present and ranged from 13 to 42 minutes with animals present. A T_{90} value of 12 minutes was selected for the 2-week and 3-month studies and 14 minutes for the 2-year studies.

The uniformity of propargyl alcohol vapor concentration in the inhalation exposure chambers without animals present was evaluated before the 3-month and 2-year studies began; concentration uniformity with animals present in the chambers was measured once during the 2-week studies, once during the 3-month studies, and approximately quarterly during the 2-year studies. The vapor concentration was measured using the on-line gas chromatograph (system D, Table I1) with the stream-selection valve fixed in one position to allow continuous monitoring from a single input line. During the 2-week study and prior to the 3-month and 2-year studies, concentrations were measured at 12 chamber positions, one in front and one in back for each of the six possible animal cage unit positions per chamber. During the 3-month and 2-year studies, concentrations were measured at the regular monitoring port and from sample ports at levels where animals were present. Chamber concentration uniformity was maintained throughout the studies.

The persistence of propargyl alcohol in the chambers after vapor delivery ended was determined by monitoring the postexposure vapor concentration in the 500 ppm chambers in the 2-week studies, the 64 ppm chambers in the 3-month studies, and the 64 ppm (rats) and 32 ppm (mice) chambers in the 2-year studies, with (all studies) and without (3-month and 2-year studies) animals present in the chambers. In the 2-week studies, the concentration decreased to 1% of the target concentration within 23 minutes. In the 3-month studies, the concentration decreased to 1% of the target concentration within 21 minutes without animals present and within 146 minutes with animals present. In the 2-year studies, the concentration decreased to 1% of the target concentration within 21 (rats) and 17 (mice) minutes without animals present and within 277 (rats) and 93 (mice) minutes with animals present.

Samples of the test atmosphere from the distribution lines and low and high exposure concentration chambers were collected prior to the 3-month and 2-year studies and also at the beginning and end of one generation day during the 2-week, 3-month, and 2-year studies; the atmosphere samples were collected with adsorbent gas sampling tubes containing silica gel (ORBO-52), followed by a tube containing activated coconut charcoal (ORBO-32) and diluted or extracted with 15:85 methanol:methylene chloride. All of the samples were analyzed using GC by a system similar to system B (Table I1) to measure the stability and purity of propargyl alcohol in the generation and delivery system. To assess whether impurities or degradation products co-eluted with propargyl alcohol or the solvent, a second GC analysis of the samples was performed using a column capable of resolving compounds with similar boiling points and polarities (system F). Five months into the 2-year study, the exposure generation pump had to be replaced. To confirm the stability and purity of the test chemical using the new pump, samples exposed to the pump overnight and vapor samples from the distribution line were collected and analyzed as previously described. In conjunction with the stability and purity measurements described above, the relative purity of propargyl alcohol in the generator reservoir was measured using GC by systems A (comparison to an independent reference standard) and B (area percent purity). No evidence of degradation of propargyl alcohol was noted in any part of the exposure system.

The concentration of formaldehyde in the exposure chambers was determined from triplicate samples collected from the distribution line in 2,4-dinitrophenylhydrazine-coated silica adsorbent sampling tubes (LpDNPH S10; Supelco), using HPLC by a method previously described for purity. Formaldehyde was present at 0.2% (2-week and 3-month studies) and 0.3% (2-year studies) relative to propargyl alcohol.

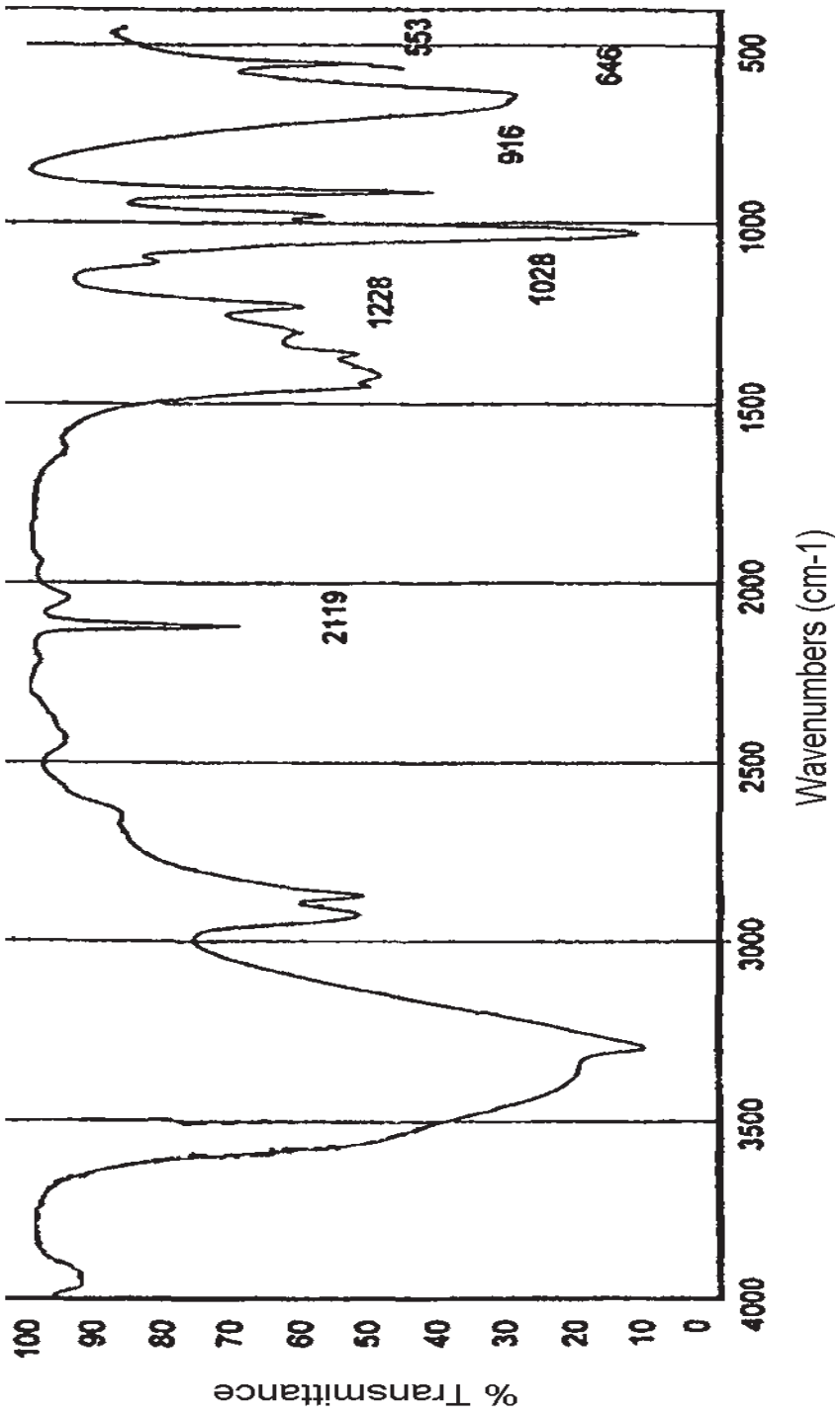

FIGURE I1
Infrared Absorption Spectrum of Propargyl Alcohol

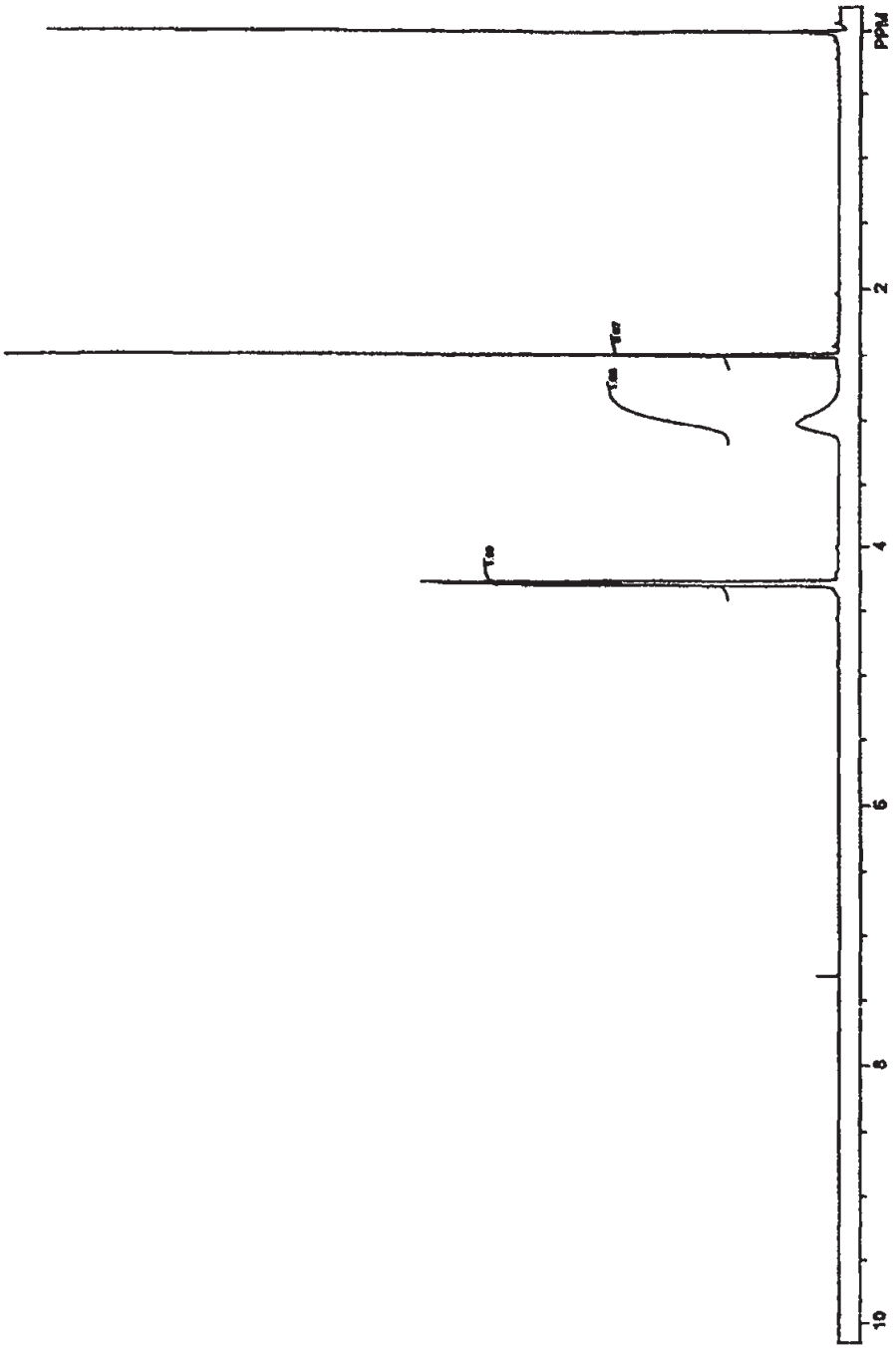

FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of Propargyl Alcohol

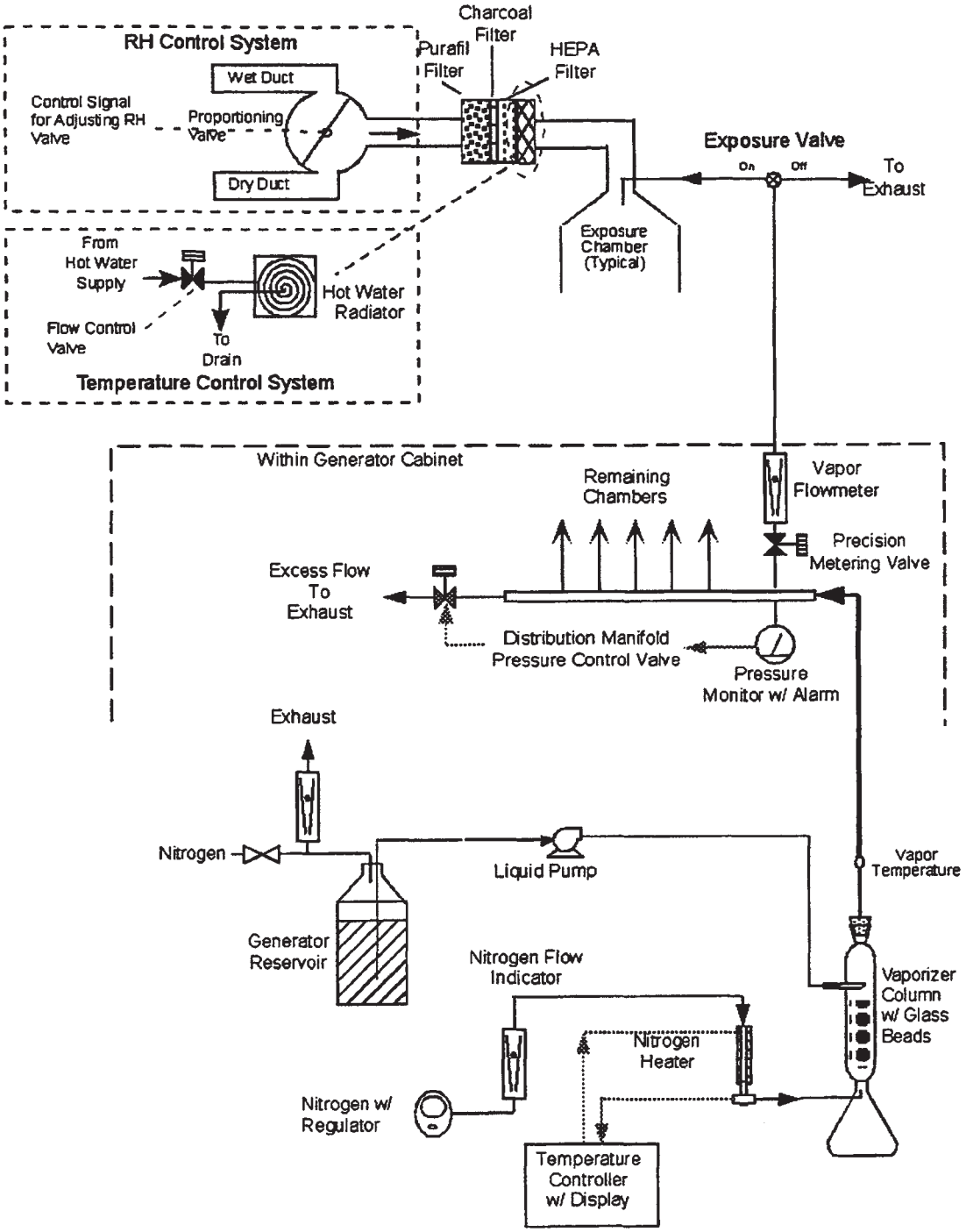

FIGURE I3
Schematic of the Generation and Delivery System in the Inhalation Studies of Propargyl Alcohol

TABLE I1
Gas Chromatography Systems Used in the Inhalation Studies of Propargyl Alcohol[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Mass spectrometry	DB-Wax 30 m × 0.32 mm, 0.5-µm film thickness (Hewlett-Packard, Palo Alto, CA)	Helium at 30 cm/sec	50° C for 5 minutes, then 50° C to 200° C, at 10° C/minute, held 10 minutes
System B			
Flame ionization	DB-Wax ETR 30 m × 0.25 mm, 0.25-µm film thickness (J&W Scientific, Folsom, CA)	Helium at 24 psi head pressure	60° C for 1 minute, then 8° C/ minute to 120° C
System C			
Flame ionization	Wax-ETR 30 m × 0.25 mm, 0.25-µm film thickness (J&W Scientific)	Helium at 24 psi head pressure	45° C for 1 minute, then 5° C/ minute to 260° C
System D			
Flame ionization	DB-Wax ETR 15 m × 0.53 mm, 1.0-µm film thickness (J&W Scientific)	Nitrogen at 8 psi head pressure	Isothermal at 70° C
System E			
Flame ionization	DB-Wax ETR 30 m × 0.53 mm, 1.0-µm film thickness (J&W Scientific)	Helium at 6 psi head pressure	50° C for 1 minute (2-week) or 0.5 minute (3-month and 2-year), then 10° C/minute (2-week) or 8° C/minute (3-month and 2-year) to 120° C, held 1 minute
System F			
Flame ionization	Restek RTX-5, 30 m × 0.25 mm, 1.0-µm film thickness (Restek, Bellefonte, PA) or DB-5 30 mm × 0.25 mm, 1.0-µm film thickness (J&W Scientific)	Helium at 24 psi head pressure	45° C for 1 minute, then 5° C/ minute to 260° C, held 5 minutes

[a] The gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA)

TABLE I2
Summary of Chamber Concentrations in the 2-Week Inhalation Studies of Propargyl Alcohol

Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers		
31.3	211	30.9 ± 1.0
62.5	209	62.1 ± 2.0
125	209	123 ± 4
250[b]	19	251 ± 11
500[b]	18	508 ± 14
Mouse Chambers		
31.3	229	30.9 ± 1.0
62.5	227	62.1 ± 1.9
125[c]	37	124 ± 4
250[b]	19	251 ± 11
500[b]	18	508 ± 14

[a] Mean ± standard deviation
[b] Includes data only from the first day of exposure
[c] Includes data only through the second day of exposure

TABLE I3
Summary of Chamber Concentrations in the 3-Month Inhalation Studies of Propargyl Alcohol

Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers		
4	1,095	4.01 ± 0.12
8	1,111	7.99 ± 0.22
16	1,122	16.0 ± 0.4
32	1,124	32.0 ± 0.7
64	1,133	64.2 ± 2.6
Mouse Chambers		
4	1,131	4.01 ± 0.12
8	1,149	8.00 ± 0.21
16	1,160	16.0 ± 0.4
32	1,162	32.0 ± 0.7
64	1,171	64.2 ± 2.6

[a] Mean ± standard deviation

TABLE I4
Summary of Chamber Concentrations in the 2-Year Inhalation Studies of Propargyl Alcohol

	Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers			
	16	7,448	16.1 ± 0.5
	32	7,663	32.0 ± 1.1
	64	7,761	64.3 ± 2.3
Mouse Chambers			
	8	7,401	8.03 ± 0.22
	16	7,416	16.1 ± 0.4
	32	7,525	32.1 ± 1.0

[a] Mean ± standard deviation

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE J1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE J2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

Amount	Source	
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE J3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.7 ± 0.60	13.7 – 15.7	23
Crude fat (% by weight)	8.1 ± 0.27	7.6 – 8.6	23
Crude fiber (% by weight)	9.0 ± 0.39	8.1 – 9.9	23
Ash (% by weight)	5.2 ± 0.25	4.8 – 5.8	23
Amino Acids (% of total diet)			
Arginine	0.750 ± 0.048	0.670 – 0.850	15
Cystine	0.225 ± 0.025	0.150 – 0.250	15
Glycine	0.701 ± 0.039	0.620 – 0.750	15
Histidine	0.365 ± 0.090	0.310 – 0.680	15
Isoleucine	0.533 ± 0.038	0.430 – 0.590	15
Leucine	1.077 ± 0.059	0.960 – 1.150	15
Lysine	0.703 ± 0.125	0.310 – 0.830	15
Methionine	0.402 ± 0.049	0.260 – 0.460	15
Phenylalanine	0.615 ± 0.035	0.540 – 0.660	15
Threonine	0.492 ± 0.040	0.430 – 0.590	15
Tryptophan	0.135 ± 0.018	0.110 – 0.160	15
Tyrosine	0.378 ± 0.048	0.280 – 0.460	15
Valine	0.658 ± 0.043	0.550 – 0.710	15
Essential Fatty Acids (% of total diet)			
Linoleic	3.90 ± 0.256	3.49 – 4.54	15
Linolenic	0.30 ± 0.035	0.21 – 0.35	15
Vitamins			
Vitamin A (IU/kg)	4,763 ± 839	3,060 – 6,920	23
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 – 110.0	15
Thiamine (ppm)[b]	7.7 ± 1.13	5.9 – 9.2	23
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm)[b]	9.21 ± 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 ± 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B_{12} (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm)[b]	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	1.006 ± 0.050	0.873 – 1.110	23
Phosphorus (%)	0.607 ± 0.034	0.555 – 0.701	23
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.37 ± 0.151	0.18 – 0.50	23
Cadmium (ppm)	0.05 ± 0.016	0.04 – 0.09	23
Lead (ppm)	0.07 ± 0.027	0.05 – 0.17	23
Mercury (ppm)	<0.02		23
Selenium (ppm)	0.22 ± 0.056	0.14 – 0.36	23
Aflatoxins (ppb)	<5.00		23
Nitrate nitrogen (ppm)[c]	14.8 ± 3.70	7.88 – 23.2	23
Nitrite nitrogen (ppm)[c]	<0.61		23
BHA (ppm)[d]	<1.0		23
BHT (ppm)[d]	<1.0		23
Aerobic plate count (CFU/g)	15.0 ± 15.0	10.0 – 70.0	23
Coliform (MPN/g)	3.0 ± 0.0	3.0 – 3.0	23
Escherichia coli (MPN/g)	<10		23
Salmonella (MPN/g)	Negative		23
Total nitrosoamines (ppb)[e]	4.3 ± 1.53	2.3 – 8.4	23
N-Nitrosodimethylamine (ppb)[e]	2.8 ± 1.42	1.2 – 6.9	23
N-Nitrosopyrrolidine (ppb)[e]	1.5 ± 0.59	0.9 – 3.1	23
Pesticides (ppm)			
α-BHC	<0.01		23
β-BHC	<0.02		23
γ-BHC	<0.01		23
δ-BHC	<0.01		23
Heptachlor	<0.01		23
Aldrin	<0.01		23
Heptachlor epoxide	<0.01		23
DDE	<0.01		23
DDD	<0.01		23
DDT	<0.01		23
HCB	<0.01		23
Mirex	<0.01		23
Methoxychlor	<0.05		23
Dieldrin	<0.01		23
Endrin	<0.01		23
Telodrin	<0.01		23
Chlordane	<0.05		23
Toxaphene	<0.10		23
Estimated PCBs	<0.20		23
Ronnel	<0.01		23
Ethion	<0.02		23
Trithion	<0.05		23
Diazinon	<0.10		23
Methyl chlorpyrifos	0.107 ± 0.064	0.020 – 0.259	23
Methyl parathion	<0.02		23
Ethyl parathion	<0.02		23
Malathion	0.340 ± 0.485	0.020 – 1.850	23
Endosulfan I	<0.01		23
Endosulfan II	<0.01		23
Endosulfan sulfate	<0.03		23

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All values were corrected for percent recovery.

APPENDIX K
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from five male and five female chamber control rats and mice at the end of the 2-week and 3-month studies, from five male and five female sentinel rats and mice at week 1 in the 3-month and 2-year studies, from four or five male and four or five female sentinel rats and mice at 6, 12, and 18 months, and from five male and five female 64 ppm rats and 32 ppm mice at the end of the 2-year studies. Fecal samples were taken from sentinel mice at 18 months. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and analyzed at the study laboratory or sent to BioReliance Corporation (Rockville, MD) for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
2-Week Study	
ELISA	
H-1 (Toolan's H-1 virus)	Study termination
KRV (Kilham rat virus)	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination
3-Month Study	
ELISA	
H-1	1 week
KRV	1 week
Mycoplasma arthritidis	Study termination
M. pulmonis	1 week and study termination
PVM	1 week and study termination
RCV/SDA	1 week and study termination
Sendai	1 week and study termination
Immunofluorescence Assay	
Parvovirus	Study termination
PVM	Study termination

Method and Test	Time of Analysis
RATS (continued)	
2-Year Study	
ELISA	
H-1	1 week
KRV	1 week
M. arthritidis	Study termination
M. pulmonis	1 weeks and study termination
PVM	1 week, 6, 12, and 18 months, study termination
RCV/SDA	1 week, 6, 12, and 18 months, study termination
Sendai	1 week, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	6, 12, and 18 months, study termination
Sendai	12 months
MICE	
2-Week Study	
ELISA	
GDVII (mouse encephalomyelitis virus)	Study termination
MHV (mouse hepatitis virus)	Study termination
MVM (minute virus of mice)	Study termination
M. pulmonis	Study termination
PVM	Study termination
Sendai	Study termination
3-Month Study	
ELISA	
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
GDVII	1 week and study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
Mouse adenoma virus	Study termination
MHV	1 week and study termination
MVM	1 week
M. arthritidis	Study termination
M. pulmonis	1 week and study termination
PVM	1 week and study termination
Reovirus 3	Study termination
Sendai	1 week and study termination
Immunofluorescence Assay	
MCMV (mouse cytomegalovirus)	Study termination
Parvovirus	Study termination

Method and Test	Time of Analysis
MICE (continued)	
2-Year Study	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM	6, 12, and 18 months, study termination
GDVII	1 week, 6, 12, and 18 months, study termination
LCM	6, 12, and 18 months, study termination
Mouse adenoma virus	6, 12, and 18 months, study termination
MHV	1 week, 6, 12, and 18 months, study termination
MVM	1 week
M. arthritidis	Study termination
M. pulmonis	1 week and study termination
PVM	1 week, 6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	1 week, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
EDIM	Study termination
Helicobacter spp. (fecal)	18 months
Mouse adenoma virus	18 months, study termination
MCMV	Study termination
Parvovirus	6, 12, and 18 months, study termination
Reovirus 3	Study termination

RESULTS

All test results were negative.

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