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

# NTP Technical Report on the Toxicology and Carcinogenesis Studies of Cumene (CAS No. 98-82-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies)

NTP TR 542

FEBRUARY 2009

NTP TECHNICAL REPORT

ON THE

# TOXICOLOGY AND CARCINOGENESIS

# STUDIES OF CUMENE

## (CAS NO. 98-82-8)

# IN F344/N RATS AND B6C3F1 MICE

## (INHALATION STUDIES)

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

February 2009

NTP TR 542

NIH Publication No. 09-5885

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.

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# CONTENTS

## SUMMARY

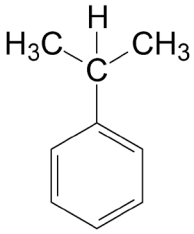
**Background**
Cumene occurs naturally in petroleum and is used as a solvent, in gasoline and diesel fuels, and as the principal chemical in the production of phenol and acetone. We studied cumene to determine if it caused cancer in rats or mice.

**Methods**
We exposed groups of 50 male and female rats and mice to air containing cumene 6 hours per day for 2 years. Rats and male mice were exposed to concentrations of 250, 500, or 1,000 parts per million (ppm) of cumene in air, and female mice were exposed to concentrations of 125, 250, or 500 ppm. Similar groups of 50 animals were exposed to clean air in the same inhalation chambers 6 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 cumene exhibited hyperplasia of the epithelial tissues of the nose, and exposed male and female mice experienced metaplasia and hyperplasia of the lung. Male mice also had nonneoplastic lesions in the forestomach and liver. Adenomas of the respiratory epithelium of the nose were observed in male and female rats, and male rats had increased incidences of renal tubule adenoma or carcinoma (combined) and interstitial cell adenoma of the testis. Adenomas and carcinomas of the lung were markedly increased in male and female mice exposed to cumene. The rate of liver neoplasms was also increased in exposed female mice, and a few hemangiosarcomas of the spleen and follicular cell adenomas of the thyroid gland were seen in male mice exposed to the highest concentration of cumene.

**Conclusions**
We conclude that the increased occurrences of adenomas of the epithelium of the nose in male and female rats, of renal tubule adenoma or carcinoma (combined), of adenomas and carcinomas of the lung in male and female mice, and of liver neoplasms in female mice were caused by exposure to cumene. The occurrence of interstitial cell adenoma of the testis in male rats and hemangiosarcomas of the spleen and follicular cell adenomas of the thyroid gland in male mice may also have been associated with exposure to cumene.

# ABSTRACT



**CUMENE**

CAS No. 98-82-8

Chemical Formula: $C_9H_{12}$ Molecular Weight: 120.19

**Synonyms:** Cumol; isopropylbenzene; isopropylbenzol; (1-methyl/ethyl)benzene; 2-phenylpropane

Cumene is produced in a modified Friedel-Crafts reaction process that uses acidic catalysts to alkylate benzene with propylene. Cumene is the principal chemical used in the production of phenol and acetone. Cumene is used to produce acetophenone, $\alpha$-methylstyrene, diisopropylbenzene, and dicumylperoxide; as a thinner; as a constituent of some petroleum-based solvents; in gasoline blending, diesel fuel, and high-octane aviation fuel; and as a raw material for peroxides and oxidation catalysts. Because cumene is a good solvent for fats and resins, it has been suggested as a replacement for benzene in many industrial applications. Cumene occurs naturally in petroleum and in a variety of foodstuffs. Cumene was nominated for study by the NIEHS because of its high production volume, presence in gasoline and other fuels, potential for human exposure, and lack of existing carcinogenicity test data. Male and female F344/N rats and B6C3F1 mice were exposed to cumene (greater than 99.9% pure) by inhalation for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium,* rat bone marrow, and mouse peripheral blood.

## 2-Week Study in Rats

Groups of five male and five female rats were exposed to cumene vapor at concentrations of 0, 250, 500, 1,000, 2,000, or 4,000 ppm, 6 hours plus $T_{90}$ (12 minutes) per day, 5 days per week for 16 days. All rats exposed to 4,000 ppm died on day 1, and two male and three female rats exposed to 2,000 ppm died by day 4. Mean body weights of 2,000 ppm rats were significantly less than those of the chamber controls. Rats exposed to 2,000 ppm that died early were severely lethargic following daily exposure. Liver and kidney weights of all exposed groups were increased. Accumulation of minimal to mild hyaline droplets was observed in the renal tubular cortex of males exposed to concentrations of 250 to 2,000 ppm.

## 2-Week Study in Mice

Groups of five male and five female mice were exposed to cumene vapor at concentrations of 0, 250, 500, 1,000, 2,000, or 4,000 ppm, 6 hours plus $T_{90}$ (12 minutes) per

day, 5 days per week for 17 days. All mice exposed to 4,000 ppm died on day 1; all mice exposed to 2,000 ppm died on day 2, and four female mice exposed to 1,000 ppm died by day 4. Mean body weights of all exposed groups were similar to those of the chamber controls. Mice exposed to 2,000 ppm were severely lethargic after the first exposure. The four female mice exposed to 1,000 ppm that died early exhibited signs of lethargy and ataxia. Liver weights, both relative and absolute, were increased in all groups of surviving males and in the 250 and 500 ppm female groups.

## 3-Month Study in Rats

Groups of 10 male and 10 female rats were exposed to cumene vapor at concentrations of 0, 62.5, 125, 250, 500, or 1,000 ppm, 6 hours plus $T_{90}$ (12 minutes) per day, 5 days per week for 14 weeks. Additional clinical pathology groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days. All rats survived to the end of the study, and mean body weights of all exposed groups were similar to those of the chamber controls. Kidney and liver weights of 250 ppm or greater males and liver weights of 1,000 ppm females were significantly greater than those of the chamber controls. There were significant differences between exposed and chamber control females in the relative length of time spent in the estrous stages. The amount of $\alpha$2u-globulin in the right kidneys was significantly increased in male rats exposed to 125 ppm or greater. The incidences of medullary granular casts in males exposed to 250 ppm or greater were significantly increased. The severities of renal tubule cortex hyaline droplet accumulation and regeneration increased with increasing exposure concentration in male rats.

## 3-Month Study in Mice

Groups of 10 male and 10 female mice were exposed to cumene vapor at concentrations of 0, 62.5, 125, 250, 500, or 1,000 ppm, 6 hours plus $T_{90}$ (12 minutes) per day, 5 days per week for 14 weeks. Eight 1,000 ppm females died during week 1 of the study. Mean body weights of males exposed to 500 or 1,000 ppm were significantly less than those of the chamber controls. The eight 1,000 ppm female mice that died during the first week of the study exhibited clinical signs of acute toxicity, including lethargy or ataxia. Liver weights of mice exposed to 500 or 1,000 ppm were significantly increased. The weight of the cauda epididymis and the spermatid count were significantly decreased in 1,000 ppm males.

## 2-Year Study in Rats

Groups of 50 male and 50 female rats were exposed to cumene vapor at concentrations of 0, 250, 500, or 1,000 ppm, 6 hours plus $T_{90}$ (12 minutes) per day, 5 days per week for 105 weeks. Survival of all exposed groups of rats was similar to that of the chamber controls. Mean body weights of 1,000 ppm females were slightly less than those of the chamber controls during the second year of the study but were similar to the chamber controls at the end of the study.

Incidences of adenoma of the respiratory epithelium in the nose occurred with a positive trend in males and were significantly increased in all exposed groups of males and in 250 ppm females. Incidences of hyperplasia of basal cells in the olfactory epithelium in the nose of all exposed groups and hyperplasia of the respiratory epithelium in the nose of all exposed groups of males and 1,000 ppm females were significantly increased.

The incidences of renal tubule adenoma in all exposed groups of males, renal tubule carcinoma in 500 and 1,000 ppm males, and renal tubule adenoma or carcinoma (combined) in all exposed groups of males were increased; the difference from chamber controls for the combined incidence was significant at 500 ppm. The incidences of hyperplasia of the renal tubule and transitional epithelium of the renal pelvis in 500 and 1,000 ppm males and mineralization of the renal papilla in all exposed groups of males were significantly greater than those of the chamber controls.

The incidence of interstitial cell adenoma (including bilateral) of the testis was significantly increased in 1,000 ppm male rats, and there was a positive trend in the incidences across all groups.

## 2-Year Study in Mice

Groups of 50 male and 50 female mice were exposed to cumene vapor at concentrations of 0, 125 (female mice only), 250, 500, or 1,000 (male mice only) ppm,

6 hours plus $T_{90}$ (12 minutes) per day, 5 days per week for 105 weeks. An exposure concentration-related decrease in survival occurred in male mice, and the survival of 1,000 ppm males was significantly less than that of the chamber controls. Mean body weights of 1,000 ppm males were generally less than those of the chamber controls after week 8 of the study, and those of 500 ppm females were less from week 28 until week 76 of the study.

The incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma or carcinoma (combined) in all exposed groups of mice occurred with positive trends and were significantly greater than those in the chamber controls. The incidences of alveolar epithelial bronchiole metaplasia and bronchiole hyperplasia were significantly increased in all exposed groups of mice. *p53* and K-*ras* mutations were found in 52% and 87% of lung neoplasms in exposed mice compared to 0% and 14% in the chamber controls, respectively.

In female mice, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) occurred with positive trends and were significantly increased in the 500 ppm group. In male mice, there were significant increases in the incidences of eosinophilic foci of the liver.

The incidences of hemangiosarcoma in the spleen and of follicular cell adenoma in the thyroid gland were significantly increased in 1,000 ppm male mice.

In the nose, the incidences of olfactory epithelium atrophy, basal cell hyperplasia of the olfactory epithelium, atypical hyperplasia of the olfactory epithelium, hyperplasia of olfactory epithelium glands, and suppurative inflammation were generally significantly increased in 500 and 1,000 ppm males and 500 ppm females. The incidence of squamous metaplasia of the respiratory epithelium was significantly increased in 500 ppm females. The incidence of basal cell hyperplasia was also significantly increased in 250 ppm females.

The incidences of epithelial hyperplasia of the forestomach in the 500 and 1,000 ppm groups of males and the incidences of ulceration and inflammation of the forestomach in 1,000 ppm males were significantly increased.

## GENETIC TOXICOLOGY

Cumene was not mutagenic in *S. typhimurium* strain TA97, TA98, TA100, or TA1535, when tested with and without liver S9 activation enzymes. Cumene induced small, but significant, increases in micronucleated polychromatic erythrocytes in bone marrow of male rats treated by intraperitoneal injection. In contrast, no increase in micronucleated erythrocytes was observed in peripheral blood of male or female mice exposed to cumene by inhalation for 3 months.

## CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of cumene in male F344/N rats based on increased incidences of respiratory epithelial adenoma in the nose and renal tubule adenoma or carcinoma (combined). Increased incidences of interstitial cell adenoma of the testis may have been related to exposure to cumene. There was *some evidence of carcinogenic activity* of cumene in female F344/N rats based on the incidences of respiratory epithelium adenoma in the nose. There was *clear evidence of carcinogenic activity* of cumene in male B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. The increased incidences of hemangiosarcoma in the spleen and follicular cell adenoma in the thyroid gland in male mice may have been related to cumene exposure. There was *clear evidence of carcinogenic activity* of cumene in female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. Increased incidences of hepatocellular adenoma or carcinoma (combined) in female mice were also considered to be related to exposure to cumene.

Exposure of male rats to cumene resulted in nonneoplastic lesions of the kidney characteristic of $\alpha$2u-globulin accumulation. Exposure to cumene resulted in nonneoplastic lesions in the nose of male and female rats; the lung, nose, liver, and forestomach of male mice; and the lung and nose of female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Report 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 Cumene**

| | Male F344/N Rats | Female F344/N Rats | Male B6C3F1 Mice | Female B6C3F1 Mice |
|---|---|---|---|---|
| **Concentrations in air** | 0, 250, 500, or 1,000 ppm | 0, 250, 500, or 1,000 ppm | 0, 250, 500, or 1,000 ppm | 0, 125, 250, or 500 ppm |
| **Body weights** | Exposed groups similar to the chamber control group | Exposed groups similar to the chamber control group | 1,000 ppm males less than the chamber control group | Exposed groups similar to the chamber control group |
| **Survival rates** | 26/50, 23/50, 27/50, 24/50 | 21/50, 27/50, 31/50, 32/50 | 38/50, 34/50, 30/50, 23/50 | 37/50, 36/50, 39/50, 35/50 |
| **Nonneoplastic effects** | Nose: olfactory epithelium, hyperplasia, basal cell (0/50, 19/50, 27/49, 26/50); respiratory epithelium, hyperplasia (0/50, 15/50, 16/49, 23/50)<br><br>Kidney: renal tubule, hyperplasia (0/50, 3/50, 8/50, 6/50); papilla, mineralization (5/50, 35/50, 44/50, 41/50); pelvis, transitional epithelium, hyperplasia (3/50, 5/50, 14/50, 15/50) | Nose: olfactory epithelium, hyperplasia, basal cell (0/50, 14/48, 25/50, 31/50); respiratory epithelium, hyperplasia (0/50, 0/48, 4/50, 6/50) | Lung: alveolar epithelium, bronchiole, metaplasia (5/50, 43/50, 42/50, 39/50); bronchiole, hyperplasia (0/50, 11/50, 17/50, 18/50)<br><br>Nose: olfactory epithelium, atrophy (4/50, 13/50, 11/49, 38/48); olfactory epithelium, hyperplasia, basal cell (0/50, 0/50, 15/49, 33/48); olfactory epithelium, hyperplasia, atypical (0/50, 0/50, 5/49, 11/48); olfactory epithelium, glands, hyperplasia (3/50, 11/50, 9/49, 23/48); inflammation, suppurative (2/50, 2/50, 9/49, 6/48)<br><br>Liver: eosinophilic focus (6/50, 5/50, 16/50, 14/50)<br><br>Forestomach: epithelium, hyperplasia (2/50, 7/50, 8/50, 13/49); ulcer (1/50, 4/50, 6/50, 6/49); inflammation (0/50, 2/50, 1/50, 5/49) | Lung: alveolar epithelium, bronchiole, metaplasia (0/50, 42/50, 49/50, 47/50); bronchiole, hyperplasia (0/50, 17/50, 10/50, 14/50)<br><br>Nose: olfactory epithelium, atrophy (4/50, 11/50, 9/50, 18/50); olfactory epithelium, hyperplasia, basal cell (0/50, 1/50, 11/50, 25/50); olfactory epithelium, hyperplasia, atypical (0/50, 0/50, 2/50, 10/50); olfactory epithelium, glands, hyperplasia (1/50, 4/50, 4/50, 11/50); respiratory epithelium, metaplasia, squamous (0/50, 0/50, 1/50, 6/50); inflammation, suppurative (0/50, 1/50, 3/50, 7/50) |

## Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Cumene

| | Male F344/N Rats | Female F344/N Rats | Male B6C3F1 Mice | Female B6C3F1 Mice |
|---|---|---|---|---|
| **Neoplastic effects** | Nose: respiratory epithelium, adenoma (0/50, 7/50, 18/49, 10/50)<br><br>Kidney: renal tubule, adenoma or carcinoma (2/50, 5/50, 8/50, 7/50) | Nose: respiratory epithelium, adenoma (0/50, 5/48, 4/50, 3/50) | Lung: alveolar/ bronchiolar adenoma (13/50, 31/50, 31/50, 29/50); alveolar/bronchiolar carcinoma (9/50, 19/50, 32/50, 33/50); alveolar/bronchiolar adenoma or carcinoma (19/50, 38/50, 42/50, 43/50) | Lung: alveolar/ bronchiolar adenoma (1/50, 26/50, 36/50, 38/50); alveolar/bronchiolar carcinoma (3/50, 16/50, 20/50, 34/50); alveolar/bronchiolar adenoma or carcinoma (4/50, 31/50, 42/50, 46/50)<br><br>Liver: hepatocellular adenoma (18/50, 23/50, 27/50, 29/50); hepatocellular adenoma or carcinoma (25/50, 26/50, 29/50, 36/50) |
| **Equivocal findings** | Testes: interstitial cell, adenoma (18/50, 14/50, 13/50, 9/50); bilateral, interstitial cell, adenoma (18/50, 24/50, 27/50, 37/50); interstitial cell and bilateral interstitial cell adenoma (36/50, 38/50, 40/50, 46/50) | | Spleen: hemangiosarcoma (0/50, 0/50, 0/49, 4/50)<br><br>Thyroid gland: follicular cell, adenoma (0/50, 0/50, 0/49, 3/50) | |
| **Level of evidence of carcinogenic activity** | Clear evidence | Some evidence | Clear evidence | Clear evidence |
| **Genetic toxicology** | | | | |
| *Salmonella typhimurium* gene mutations: | Negative in strains TA97, TA98, TA100, and TA1535, with and without S9 | | | |
| Micronucleated erythrocytes | | | | |
| Rat bone marrow *in vivo*: | Positive | | | |
| Mouse peripheral blood *in vivo*: | Negative | | | |

## 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 cumene on May 16, 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 16, 2007, the draft Technical Report on the toxicology and carcinogenicity study of cumene 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. P.C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of cumene by describing the uses and human exposure possibilities of the chemical, the genetic toxicity profile, the study design, and the survival, body weights, and neoplastic and nonneoplastic lesions observed in the 3-month and 2-year studies. Dr. D.E. Malarkey, NIEHS, described the histopathologic details of the lesions seen in the respiratory tract and some of the supplemental molecular pathology findings. The proposed conclusions were *clear evidence of carcinogenic activity* of cumene in male F344/N rats, *some evidence of carcinogenic activity* of cumene in female F344/N rats, and *clear evidence of carcinogenic activity* of cumene in male and female B6C3F1 mice.

Dr. Mirsalis, the first principal reviewer, had no major scientific criticisms. He felt unconvinced that the rat micronucleus data showed a truly positive response.

Dr. Walker, the second principal reviewer, agreed that the micronucleus response was at best equivocal. He felt the study was well performed and especially liked the inclusion of K-*ras* and *p53* mutation analyses for the lung neoplasms.

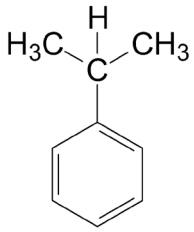
Dr. Pino, the third principal reviewer, inquired about a statistically significant increase in interstitial cell adenomas of the testes in male rats that was not included in the results section of the report. He asked about the reason for the distinction between clear and some evidence for the nasal adenomas in male and female rats.

Dr. Crump noted two other sites in male mice for which significant occurrences of tumors were not included in the results or conclusions: hemangiosarcomas of the spleen and follicular cell adenomas of the thyroid gland. He also queried whether the kidney tumors in male rats merited a classification of clear evidence.

Dr. Kerkvliet said that a written response had been submitted by the American Chemistry Council and that an oral presentation would be given by Dr. George Kruzam. Dr. Kruzam noted that in the introduction of the report cumene was incorrectly identified as being used in styrene synthesis. He suggested that the abstract of the report include a statement about the relevance of the findings to human disease and include genotoxicity results from the literature.

In consideration of the conclusion statements, Dr. Walker suggested that a statement be added indicating the association of the nonneoplastic kidney lesions in male rats with $\alpha$2u-globulin. Dr. Crump moved that mention be added of the three additional lesions: testicular adenoma in male rats and hemangioma of the spleen and thyroid gland follicular cell adenomas in male mice. Dr. J.R. Bucher, NIEHS, said that if these tumors were considered equivalent to equivocal evidence, they would be termed "may have been related" to chemical administration. The panel voted unanimously with seven votes to approve the inclusion of these three lesions as "may have been related" plus a statement concerning the $\alpha$2u-globulin accumulation.

# INTRODUCTION



**CUMENE**

CAS No. 98-82-8

Chemical Formula: $C_9H_{12}$ Molecular Weight: 120.19

**Synonyms:** Cumol; isopropylbenzene; isopropylbenzol; (1-methyl/ethyl)benzene; 2-phenylpropane

## CHEMICAL AND PHYSICAL PROPERTIES

Cumene is a volatile, colorless liquid with a sharp, penetrating aromatic or gas-like odor (*Merck*, 1989; Cavender, 1994; NIOSH, 2005). Cumene has a boiling point of 152.4° C (Lide, 1995), a melting point of –96.0° C (Lide, 1995), and a vapor pressure of 3.2 mm Hg at 20° C (Verschueren, 1983). Cumene is insoluble in water but is miscible with acetone, benzene, and ethanol (Lide, 1995). Cumene forms cumene hydroperoxide upon prolonged exposure to the air (NIOSH, 2005).

## PRODUCTION, USE, AND HUMAN EXPOSURE

Cumene as a pure chemical intermediate is produced in a modified Friedel-Crafts reaction process that uses acidic catalysts to alkylate benzene with propylene. The majority of cumene is manufactured with a solid phosphoric acid catalyst; the remainder is made with an aluminum chloride catalyst (Schulz *et al.*, 1993). The annual production of cumene in the United States was 4.49 billion pounds in 1993 and 5.63 billion pounds in 1995 (Anonymous, 1995; Kirschner, 1996; HSDB, 2003).

Cumene is the principal chemical used in the production of phenol and acetone via the chemical intermediate cumene hydroperoxide (the hydroperoxide is cleaved to phenol and acetone in an acidic environment). Cumene is used to produce acetophenone, $\alpha$-methylstyrene, diisopropylbenzene, and dicumylperoxide. Cumene can also be used as a thinner for paints, enamels, and lacquers; as a constituent of some petroleum-based solvents such as naphtha; in gasoline blending, diesel fuel, and high-octane aviation fuel; and as a raw material for peroxides and oxidation catalysts such as polymerization catalysts for acrylic and polyester-type resins. It is also a good solvent for fats and resins and, as such, has been suggested as a replacement for benzene in many industrial applications (Parmeggiani, 1983; Verschueren, 1983; Mannsville, 1985; *Merck*, 1989; ACGIH, 1993; Anonymous, 1993; *Hawley's*, 1993; Schulz *et al.*, 1993; HSDB, 2003).

Cumene occurs naturally in petroleum crudes and coal tar (Verschueren, 1983). It also occurs in a variety of natural substances, including essential oils from plants, marsh grasses, and a variety of foodstuffs. Trace quantities have been detected in papaya, sapodilla fruit, and Australian honey. Cumene has been detected but not quantified in fried chicken, tomatoes, Concord grapes,

cooked rice, oat groats, baked potatoes, Beaufort cheese, fried bacon, dried legumes (beans, split peas, lentils), southern pea seeds, and Zinfandel wine (HSDB, 2003).

The potential for worker exposure exists during production and processing from petroleum refining. General population exposure to cumene results from inhalation of air contaminated with cumene from evaporation of petroleum products and from cigarette smoke; additional exposure may result from ingestion of food (HSDB, 2003). Cumene is released into the environment as a result of cumene production, processing, and transport, petroleum refining and the evaporation and combustion of petroleum products, the transportation and distribution of motor fuel, and the use of a variety of products containing cumene. Cumene has been detected in air samples from Los Angeles, CA, at concentrations as high as 144 $\mu g/m^3$ and in groundwater, surface water, and drinking water (Jackson, 1985; USEPA, 1997; HSDB, 2003).

The threshold-limit value-time-weighted average recommended by the American Conference of Governmental Industrial Hygienists (2005) for cumene is 50 ppm (245 $mg/m^3$); a short-term exposure limit (STEL) has not been determined. The Occupational Safety and Health Administration permissible exposure limit is 50 ppm, with a skin designation, averaged over an 8-hour work shift; a STEL has not been determined (29 CFR, Part 1910.1000). The exposure limit recommended by the National Institute for Occupational Safety and Health for cumene is 50 ppm, with a skin notation, averaged over a 10-hour work shift (NIOSH, 2005). The United States Environmental Protection Agency (1997) has assigned cumene to carcinogen category D (not classifiable or inadequate human or animal data) and determined an inhalation reference concentration of 0.4 $mg/m^3$ using uncertainty factors to reflect a daily exposure concentration without appreciable risk of deleterious effects during a lifetime.

## ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Metabolism, disposition, and pharmacokinetic studies of cumene in rats following oral, intravenous injection, or nose-only inhalation administration have demonstrated that the chemical is well absorbed. Following absorption, a small quantity (approximately 5%) of the cumene was exhaled unchanged, but the major portion was oxidized at the benzylic carbon to 2-phenyl-2-propanol (Figure 1), with subsequent oxidation to 2-hydroxy-2-phenylpropanoic acid, 2-phenyl-1,2-propanediol, and phenyllactic acid. A minor pathway is oxidation of a methyl group to 2-phenyl-1-propanol and subsequently to 2-phenylpropanoic acid and 2-phenylmalonic acid. A minor metabolite may be phenylmalonic acid (Senczuk and Litewka, 1976; NRC, 1981; Parmeggiani, 1983; Gosselin *et al.*, 1984; Lee, 1987; Sato and Nakajima, 1987; Slauter and Jeffcoat, 1989, 1990, 1992; Ishida and Matsumoto, 1992; Cavender, 1994; USEPA, 1997). These metabolites were excreted mainly in the urine in conjugated form. Oxidation occurs in both hepatic and extrahepatic tissues, including the lung (Sato and Nakajima, 1987). Cumene does not bioaccumulate in tissues (ACGIH, 1993). There is no known biotransformation product that would suggest toxicity.

## TOXICITY

### *Experimental Animals*

Table 1 lists $LC_{50}$ and $LD_{50}$ values for cumene in rats, mice, and rabbits.

Mice exposed to cumene vapor exhibited dilation of cutaneous blood vessels, grades of central nervous system depression, narcosis, depression of respiration, and death, depending on the concentration and duration of exposure. The narcosis was characterized by slow induction and long duration relative to benzene and toluene.

When rats were dosed by repeated gastric intubation with 154, 462, or 769 mg cumene/kg body weight for 194 days, no changes in body weights, hematology, or histopathology of the liver or kidney were found, but at 462 and 769 mg/kg, increases in kidney weights were observed (ACGIH, 1993). Subcutaneous administration of 1 mL/kg daily for 2 weeks did not lower the femoral bone marrow cell population of rats (ACGIH, 1993).

In an inhalation study, exposure of rabbits to 1,323 ppm (6,496 $mg/m^3$) cumene for up to 180 days resulted in no changes in behavior or body weight gain (Fabre *et al.*, 1955). In the same study, inhalation exposure of rats to 509 ppm (2,499 $mg/m^3$) for 180 days produced a decrease in body weight gain limited to the initial part of the study and congestion of the lung, liver, spleen, kidney, and adrenal gland; higher exposure concentrations [814 ppm (3,997 $mg/m^3$) and 1,323 ppm (6,496 $mg/m^3$)]

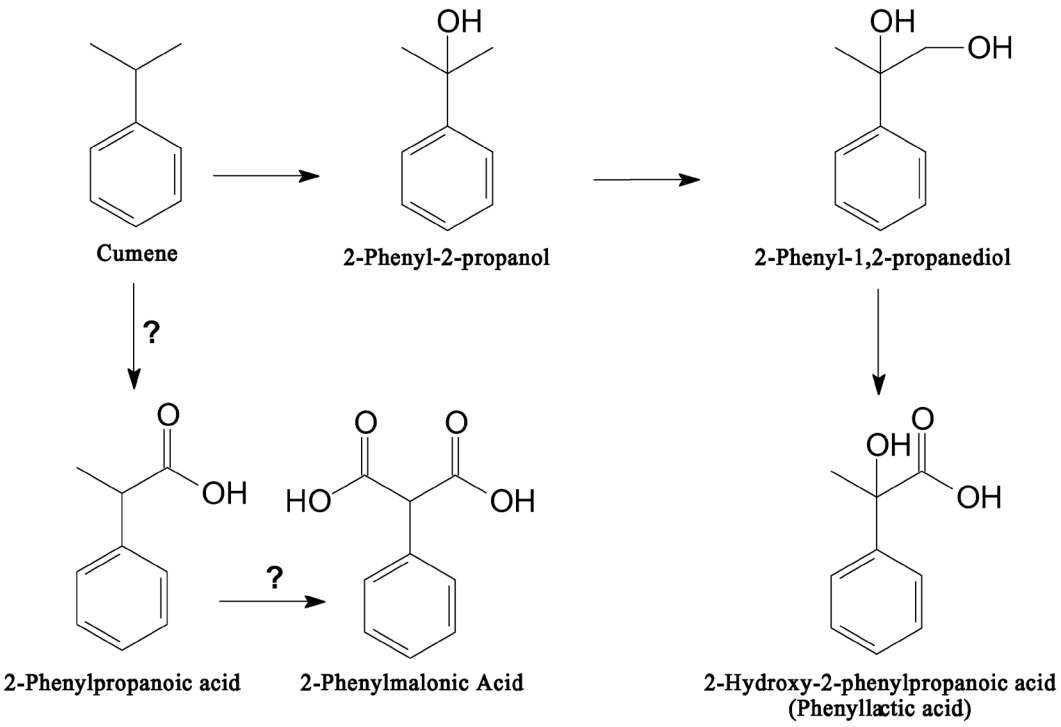



**FIGURE 1**
**Known Metabolites of Cumene**

**TABLE 1**
**Acute Toxicity Values for Cumene**[a]

| Species | Route of Administration | $LC_{50}/LD_{50}$ |
|---|---|---|
| Rats | Inhalation (4-hour) | 8,000 ppm |
| Rats | Oral | 1.4 g/kg (male) |
| Mice | Inhalation (7-hour) | 2,000 ppm |
| Mice | Oral | 12.75 g/kg |
| Rabbits | Dermal | 12.3 mL/kg |

[a] Cavender, 1994; Cushman *et al.*, 1995

killed the rats within 16 hours of exposure. Daily inhalation exposure of rats to 500 ppm for 5 months resulted in no significant changes in the peripheral blood; however, hyperemia and congestion were noted in the lung, liver, and kidney of exposed animals (ACGIH, 1993). Jenkins *et al.* (1970) used inhalation techniques to expose rats, guinea pigs, dogs, and monkeys to 244 ppm (1,195 mg/m$^3$) for 8 hours per day, 5 days per week for 30 exposures or to 3.7 ppm (18 mg/m$^3$) or 30 ppm (146 mg/m$^3$) continuously for 90 to 130 days and reported essentially negative findings.

Cushman *et al.* (1995) exposed groups of 21 male and 21 female Fischer 344 rats to cumene vapor at concentrations of 0, 100, 500, or 1,200 ppm for 6 hours per day, 5 days per week for 13 weeks. A subsequent 13-week study with a 4-week recovery period was conducted in groups of 15 male and 15 female rats at exposure concentrations of 0, 50, 100, 500, or 1,200 ppm. No exposure-related changes in body weights, mortality, a functional observation battery, auditory brain stem responses, brain measurements, or nervous system histopathology were observed. Motor activity decreases seen only in 500 and 1,200 ppm males in the first study were not replicated in the second study. The 500 and/or 1,200 ppm groups showed transient decreases in body weight gain and feed consumption, increases in water consumption, and changes in several hematologic and clinical chemistry parameters. No exposure-related ophthalmologic findings or effects on spermatogenesis occurred. Liver, kidney, and adrenal gland weights were increased in the 500 and 1,200 ppm groups. Renal proximal tubule cell hypertrophy, hyperplasia, and hyaline droplet formation were observed in 500 and 1,200 ppm males.

Daily inhalation exposure to 500 ppm cumene for 150 days was reported to induce hyperemia of the lung, liver, and kidney (species and sex not specified), but no changes occurred in the peripheral blood or bone marrow of animals exposed to 1,300 to 1,400 ppm for 180 days (Parmeggiani, 1983).

### *Humans*

Cumene is an eye, skin, and mucous membrane irritant (ACGIH, 1993; HSDB, 2003; NIOSH, 2005). Short-term exposure to cumene may cause dizziness, headache, drowsiness, slight incoordination, and unconsciousness (HSDB, 2003). Prolonged contact with liquid cumene may cause erythema or blisters (NIOSH/OSHA, 1981; Gosselin *et al.*, 1984).

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

### *Experimental Animals*

Timed-pregnant Sprague-Dawley rats (25 per group) were exposed to cumene vapor for 6 hours per day on gestational days 6 through 15 at target concentrations up to 1,200 ppm (Neeper-Bradley, 1989a). In this study, maternal toxicity was evidenced at 1,200 ppm by significant reductions in body weight gain and treatment-related clinical signs of toxicity (perioral wetness and encrustation), decreased feed consumption, and increased relative liver weight; gestational parameters and fetal body weights per litter were unaffected by the exposure. In a subsequent study, this investigator found that pregnant rabbits exposed to 2,300 ppm cumene vapor during organogenesis evidenced consistent maternal toxicity (reductions in weight gain and feed consumption and increased relative liver weight)

(Neeper-Bradley, 1989b). Gestational parameters such as the number of corpora lutea; the total, nonviable, or viable implantations per litter; sex ratio; pre- or postimplantation loss; fetal body weights per litter; and malformations exhibited no significant changes.

### *Humans*

No studies of reproductive or developmental effects of cumene in humans were found in a review of the literature.

## CARCINOGENICITY

No reports of carcinogenic effects of cumene in experimental animals or evidence of carcinogenic effects in humans in epidemiology studies or case reports were found in the literature. However, the NTP has conducted carcinogenicity studies of ethylbenzene (a structurally related chemical) administered by inhalation and reported induction of renal tubule neoplasms in male and female F344/N rats, testicular adenoma in male F344/N rats, alveolar/bronchiolar neoplasms in male B6C3F1 mice, and hepatocellular neoplasms in female B6C3F1 mice (NTP, 1999). In contrast, ethylbenzene was not carcinogenic in male or female CD (Sprague-Dawley) rats gavaged daily with 500 mg/kg, 4 to 5 days per week for 104 weeks (Maltoni *et al.*, 1985).

## GENETIC TOXICITY

There are few published reports on the mutagenicity of cumene, and the limited information available suggests that cumene is not mutagenic in standard assays. Cumene was reported to be nonmutagenic in *Salmonella typhimurium* tester strains with and without metabolic activation (Simmon *et al.*, 1977; Florin *et al.*, 1980), and results obtained with cumene in several industry-sponsored *in vitro* and *in vivo* genetic toxicity studies in mammalian cell test systems also indicated no potential for mutagenic or clastogenic activity (GLSC, 1985a,b; Curren, 1987; Putman, 1987; Yang, 1987).

As noted earlier, cumene is structurally related to ethylbenzene. NTP (1999) studies have demonstrated that ethylbenzene is not mutagenic in *S. typhimurium* tester strains with or without S9 activation enzymes (Zeiger *et al.*, 1992), but treatment of cultured mouse lymphoma L5178Y cells with ethylbenzene in the absence of S9 resulted in significantly increased mutation frequencies at the tk+/- locus (McGregor *et al.*, 1988). No induction of sister chromatid exchanges or chromosomal aberrations was observed in cultured Chinese hamster ovary cells treated with ethylbenzene with or without S9 enzymes (NTP, 1999), and no increases in the frequencies of micronucleated erythrocytes were seen in peripheral blood of male or female B6C3F1 mice exposed to ethylbenzene by inhalation for 3 months (Witt *et al.*, 2000).

## STUDY RATIONALE

Cumene was nominated for study by the NIEHS because of its high production volume, presence as a component in gasoline and other fuels, potential for human exposure, and lack of existing carcinogenicity test data. Inhalation was chosen for these studies because this is the primary route of human exposure. Cumene was studied for toxic and carcinogenic effects in rats and mice exposed by whole body inhalation for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, rat bone marrow, and mouse peripheral blood.

# MATERIALS AND METHODS

## PROCUREMENT AND CHARACTERIZATION OF CUMENE

Cumene was obtained from Sunoco, Inc. (Philadelphia, PA), in one lot (200556852) that was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were conducted by the study laboratory at Battelle Toxicology Northwest (Richland, WA), by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO), and by Chemir/Polytech Laboratories, Inc. (Maryland Heights, MO), (Appendix I). Reports on analyses performed in support of the cumene studies are on file at the National Institute of Environmental Health Sciences.

Lot 200556852 of the chemical, a colorless liquid with a sharp, penetrating, aromatic odor, was identified as cumene by the analytical chemistry laboratory and Chemir/Polytech Laboratories, Inc., using $^{1}$H-nuclear magnetic resonance spectroscopy, gas chromatography/mass spectrometry, and/or infrared spectroscopy. For lot 200556852, Karl Fischer titration indicated a water content ranging from approximately 50 to 220 ppm; elemental analyses for carbon and hydrogen were in agreement with the theoretical values for cumene. Gas chromatography by one system detected no impurities greater than 0.05%, and the purity was determined to be approximately 100%. Using gas chromatography by another system, the area percent purity for the major cumene peak was 99.9%, and no peaks were detected with an area percent greater than 0.1%. The overall purity of lot 200556852 was determined to be greater than 99.9%.

To ensure stability, the bulk chemical was stored at controlled room temperature in the original shipping containers (55-gallon metal drums). Stability was monitored by the study laboratory during the 2-week, 3-month, and 2-year studies with gas chromatography. No degradation of the bulk chemical was detected.

## VAPOR GENERATION AND EXPOSURE SYSTEM

The design of the vapor generation and exposure system was influenced by the relatively high boiling point of cumene (approximately 152° C) and the need to reach relatively high concentrations. Therefore, with the exception of individual chamber inlets, all vapor transport lines and dilution air were heated to the minimum temperature needed to move vapor to the chambers without condensation. A bulk supply of cumene was held in an 8-gallon stainless steel chemical reservoir and pumped through a preheater into the top of a heated glass column filled with glass beads to increase the surface area for evaporation. Heated nitrogen entering the column from below vaporized the chemical as it conveyed it out of the generator. Generator output was controlled by the delivery rate of the chemical metering pump.

Cumene vapor was transported to the exposure room through transport lines at an elevated temperature to prevent condensation. In the distribution manifold cabinet, the vapor was mixed with additional heated air before it entered a short vapor distribution manifold. Concentration in the manifold was determined by the chemical pump rate, nitrogen flow rate, and dilution air flow rate, all of which were monitored by the exposure operator. The pressure in the distribution manifold was fixed to ensure constant flow through the manifold and into the chambers as the flow of vapor to each chamber was adjusted.

Electronically actuated metering valves controlled the flow to each chamber. In addition, an exposure-shutoff valve, mounted in series with each chamber-metering valve, controlled vapor delivery to each chamber. Vapor was diverted to the exposure chamber exhaust until the generation system was stable and exposures were ready to proceed. To start the exposure, the valves were

opened to allow the flow of vapor to reach the chamber-metering valves and move into individual temperature-controlled delivery lines to each chamber. The vapor was then injected into the 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 condensation particle counter (Model 3022A, TSI, Inc., St. Paul, MN) was used to count the particles in all chambers before and during generation to ensure that cumene vapor, and not aerosol, was produced. No particle counts greater than 200 particles/$cm^3$ were detected.

## Vapor Concentration Monitoring

Concentrations of cumene in the exposure chambers 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 26 (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. Summaries of the chamber vapor concentrations are given in Appendix I, Tables I2 through I4.

The on-line gas chromatograph was checked throughout the day for instrument drift against an on-line standard of cumene in nitrogen supplied by a permeation tube standard generator (Kin-Tek Model 491, Kin-Tek Laboratories, Inc., 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 charcoal sampling tubes (ORBO™-101, Supelco, Bellefonte, PA), extracted with toluene containing 1,2,4-trimethylbenzene as an internal standard, and analyzed by an off-line gas chromatograph. The volumes of gas were sampled from each chamber at a constant flow rate ensured by a calibrated critical orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standard solutions of cumene and the internal standard (1,2,4-trimethylbenzene) in toluene.

## Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined with (all studies) and without (3-month and 2-year studies) 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 all studies.

The uniformity of cumene vapor concentration in the inhalation exposure chambers without animals present was evaluated before the 3-month and 2-year studies; 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 in the 2-year studies. The vapor concentration was measured using an on-line gas chromatograph. Chamber concentration uniformity was maintained throughout the studies.

The persistence of cumene in the chambers after vapor delivery ended was determined by monitoring the postexposure vapor concentration in the 4,000 ppm chambers in the 2-week studies and the 1,000 ppm chambers in the 3-month and 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 77 minutes. In the 3-month studies, the concentration decreased to 1% of the target concentration within 25 minutes without animals present and within 28 minutes with animals present. In the 2-year studies, the concentration decreased to 1% of the target concentration within 25 (rats) and 24 (mice) minutes without

animals present and within 35 (rats) and 27 (mice) minutes with animals present.

No evidence of degradation of cumene was noted in any part of the exposure system. With the exception of one peak noted in the distribution line samples taken during the 3-month studies, no impurity peaks were resolved with an area greater than 0.1% of the total peak area, and no additional impurities were detected with polar gas chromatography analyses. The relative purity of all generator reservoir and vapor trap samples exceeded 99% compared to the bulk chemical, and these samples were 99.97% pure by area percent.

## 2-Week Studies

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY). On receipt, the rats and mice were approximately 4 to 5 weeks old. Animals were quarantined for 11 days and were approximately 5 to 6 weeks old on the first day of the studies. Before the studies began, four male and six female rats and five male and five female mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At terminal sacrifice, serum was collected from five male and five female chamber control rats and mice, and serologic analyses were performed using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of five male and five female rats and mice were exposed to cumene at concentrations of 0, 250, 500, 1,000, 2,000, or 4,000 ppm, 6 hours plus $T_{90}$ (12 minutes) per day, 5 days per week for 16 (rats) or 17 (mice) days. Feed was available *ad libitum* except during exposure periods; water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded twice daily on exposure days for rats and mice. The animals were weighed initially, on days 6 and 13, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Necropsies were performed on all 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 all chamber control and 1,000 (mice only), 2,000, and 4,000 ppm animals, and tissues were examined to a no-effect level in the remaining exposure groups. Table 2 lists the tissues and organs routinely examined.

## 3-Month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to cumene 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 Laboratory Animals and Services (Germantown, NY). On receipt, the rats and mice were approximately 4 to 5 weeks old. Animals were quarantined for 11 (male rats and male and female mice) or 12 (female rats) days and were approximately 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. Serologic analyses were performed by the study laboratory on five male and five female sentinel rats and mice 3 weeks after arrival at the study laboratory and five male and five female chamber control rats and mice at the end of the studies using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were exposed to cumene at concentrations of 0, 62.5, 125, 250, 500, or 1,000 ppm, 6 hours plus $T_{90}$ (12 minutes) per day, 5 days per week for 14 weeks. Additional clinical pathology groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days. One additional exposure day was scheduled during the last exposure week to give the rats at least two consecutive days of exposure before terminal sacrifice. Feed was available *ad libitum* except during exposure periods; water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded weekly. 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 at the end of the study for hematology and clinical chemistry analyses; blood was collected from

the retroorbital sinus of mice at the end of the study for hematology analyses. Blood samples for hematology analyses were placed in tubes containing potassium EDTA. Erythrocyte, leukocyte, and platelet counts; hemoglobin; packed red cell volume; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined using an ABX Cobas Helios hematology analyzer (ABX Co., Irvine, CA). Manual hematocrit values were determined using a microcentrifuge (Heraeus Haemofuge, Germany) and a Damon/IEC capillary reader (International Equipment Company, Needham Heights, MA) for comparison to Helios values for packed cell volume. Blood smears for mice and rats were stained with Romanowsky-type aqueous stain in a Wescor 7100 Aerospray Slide Stainer (Wescor, Inc., Logan, UT). Leukocyte differential counts for rats and mice 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 containing separator gel and allowed to clot. After clot retraction occurred, the samples were centrifuged, and the serum was aliquoted for assay of serum chemistry analytes using a Roche Cobas Fara (Roche Diagnostics, Branchburg, NJ). 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 rats and mice exposed to 0, 125 (female mice only), 250, 500, or 1,000 ppm (rats and male mice). The parameters evaluated are listed in Table 2. 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, the 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. Four sperm morphology slides were prepared for each animal evaluated. 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. 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).

The left kidney was removed from all male and female core study rats at terminal necropsy, sectioned in half longitudinally, placed in a cassette, and fixed with 10% neutral buffered formalin for approximately 24 hours. After fixation, one half of the left kidney was processed and embedded in paraffin. A cross-section of small intestine was included in the embedding paraffin as a positive control for the cell proliferation study. After embedding, the left kidney was cut into three 5-µm thick sections. The first section was stained with hematoxylin and eosin for histopathology (males and females). The second section was stained with Mallory-Heidenhain for evaluation for hyaline droplets (males and females). The third section was stained with proliferating cell nuclear antigen (PCNA) complexed with avidin and biotin for determination of cell proliferation indices (males). The right kidneys of all core study male rats were frozen and stored at approximately –70° C.

For male rats, the slides stained with PCNA were evaluated to determine the number of proximal tubule cells in the *S*-phase and the labeling index. Evaluation was done using a 20× objective and an ocular grid. Counting started at the second grid in from the outer edge of the cortex of the kidney. After one grid was counted, the slide was moved toward the medulla, and every other field encountered by the grid was counted. This procedure was repeated until at least 2,000 proximal tubule nuclei (labeled and unlabeled) were counted. If 2,000 proximal tubule nuclei were counted but the entire grid had not been counted, the remainder of the grid was counted. If 2,000 proximal tubule nuclei had not been counted by the time the outer medulla was reached, the slide was moved two grids laterally, and the counting process was resumed at the second grid in from the edge of the cortex.

The frozen kidneys from core study male rats were evaluated for α2u-globulin and soluble protein. Each

right kidney was thawed; a volume of sodium/potassium phosphate buffer (pH ~7.2) equivalent to twice the recorded fresh weight of the sample was added, and the sample was homogenized for 30 to 60 seconds using an Ultra-Turrax tissue homogenizer (Tekmar Co., Cincinnati, OH). The homogenate was centrifuged at approximately 3,000 g for 15 minutes at 4° C. The protein content of each supernatant was measured in a 1:50 dilution (in phosphate-buffered saline-Tween) using the Bicinchoninic Acid Protein Assay Reagent kit (Pierce Chemical Co., Rockford, IL).

Analysis of α2u-globulin in supernatants prepared from kidney homogenates was conducted using a competitive indirect enzyme-linked immunosorbent assay (ELISA). Ascites fluid containing anti-α2u-globulin monoclonal antibodies was developed by Chemical Industry Institute of Toxicology (Research Triangle Park, NC). The amount of α2u-globulin was measured by comparing the relative fluorescent signal intensity in the study samples to that observed with known amounts of α2u-globulin present in calibration standards. Calibration standards and ELISA control standards (negative and positive) were plated in predetermined wells on 96-well microtiter plates. Calibration standards and study samples were assayed in triplicate.

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 (eyes were fixed in Davidson's solution for up to 10 days and then transferred to 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 all chamber control, 500 (female mice only), and 1,000 ppm animals, and tissues were examined to a no-effect level in the remaining exposure groups. 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 cumene at concentrations of 0, 125 (female mice only), 250, 500, or 1,000 (rats and male mice) ppm, 6 hours plus $T_{90}$ (12 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 Laboratory Animals and Services (Germantown, NY) 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 approximately 5 to 6 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, racks, and chambers were changed weekly. Cages were rotated weekly in chambers. Refer to Table 2 for more information about animal maintenance. Refer to Appendix J for information about feed composition and contaminants.

## Clinical Examinations and Pathology

All animals were observed twice daily. For rats, clinical findings were recorded every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the study. For mice, clinical findings were recorded weekly through week 13, then every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the study. Rats and mice were weighed initially, weekly for the first 13 weeks, then every 4 weeks through week 93, every 2 weeks thereafter, 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 (eyes were fixed in Davidson's solution for up to 72 hours and then transferred to 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 eye, forestomach, lung, and nose of rats and mice, the kidney of male and female rats and male mice, the urinary bladder of male rats, and the liver of 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 Cumene**

| 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<br>B6C3F1 mice | F344/N rats<br>B6C3F1 mice | F344/N rats<br>B6C3F1 mice |
| **Animal Source** | | |
| Taconic Laboratory Animals and Services (Germantown, NY) | Taconic Laboratory Animals and Services (Germantown, NY) | Taconic Laboratory Animals and Services (Germantown, NY) |
| **Time Held Before Studies** | | |
| 11 days | Rats: 11 days (males) or 12 days (females)<br>Mice: 11 days | Rats: 11 days<br>Mice: 10 days |
| **Average Age When Studies Began** | | |
| 5 to 6 weeks | 5 to 6 weeks | 5 to 6 weeks |
| **Date of First Exposure** | | |
| April 24, 2000 | Rats: July 24 (males) or 25 (females), 2000<br>Mice: July 24, 2000 | Rats: June 4, 2001<br>Mice: June 11, 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 plus $T_{90}$ (12 minutes) per day, 5 days per week for 14 weeks | 6 hours plus $T_{90}$ (12 minutes) per day, 5 days per week for 105 weeks |
| **Date of Last Exposure** | | |
| Rats: May 9, 2000<br>Mice: May 10, 2000 | Rats: October 23 (males) or 24 (females), 2000<br>Mice: October 25 (males) or 26 (females), 2000 | Rats: June 1-4, 2003<br>Mice: June 8-12, 2003 |
| **Necropsy Dates** | | |
| Rats: May 10, 2000<br>Mice: May 11, 2000 | Rats: October 24 (males) or 25 (females), 2000<br>Mice: October 26 (males) or 27 (females), 2000 | Rats: June 2-5, 2003<br>Mice: June 9-13, 2003 |
| **Average Age at Necropsy** | | |
| 8 to 9 weeks | 19 to 20 weeks | Rats: 110 to 111 weeks<br>Mice: 109 to 111 weeks |
| **Size of Study Groups** | | |
| 5 males and 5 females | Rats: 10 males and 10 females (core study), 10 males and 10 females (clinical pathology study)<br>Mice: 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 Cumene**

| 2-Week Studies | 3-Month Studies | 2-Year Studies |
|---|---|---|
| **Method of Animal Identification** | | |
| Tail tattoo | Tail tattoo | Tail tattoo |
| **Diet** | | |
| NTP-2000 irradiated pelleted diet (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum* (except during animal exposure periods); changed weekly | Same as 2-week studies | Same as 2-week studies, except wafer form |
| **Water** | | |
| Tap water (Richland, WA, 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 |
| **Cageboard** | | |
| Untreated paper cage pan liner; changed daily | Same as 2-week studies | Untreated paper cage pan liner (Techboard, Sheperd Specialty Papers, Kalamazoo, MI); changed daily |
| **Chamber Air Supply Filters** | | |
| Single HEPA (Environmental Filter, Santa Rosa, CA), changed annually; charcoal (RSE, Inc., New Baltimore, MI), new at study start; Purafil (Environmental Systems, Lynwood, WA), new at study start | Same as 2-week studies | Same as 2-week studies, except single HEPA was open stock |
| **Chambers** | | |
| Stainless steel, excreta pan at each of six levels (Lab Products, Inc., Seaford, DE); chambers changed weekly; excreta pans changed daily | Same as 2-week studies | Same as 2-week studies |
| **Chamber Environment** | | |
| Temperature: 75° ± 3° F<br>Relative humidity: 55% ± 15%<br>Room fluorescent light: 12 hours/day<br>Chamber air changes: 15 ± 2/hour | Temperature: 75° ± 3° F<br>Relative humidity: 55% ± 15%<br>Room fluorescent light: 12 hours/day<br>Chamber air changes: 15 ± 2/hour | Temperature: 75° ± 3° F<br>Relative humidity: 55% ± 15%<br>Room fluorescent light: 12 hours/day<br>Chamber air changes: 15 ± 2/hour |
| **Exposure Concentrations** | | |
| 0, 250, 500, 1,000, 2,000, and 4,000 ppm | 0, 62.5, 125, 250, 500, and 1,000 ppm | Rats: 0, 250, 500, and 1,000 ppm<br>Mice: 0, 125 (females only), 250, 500, and 1,000 (males only) ppm |

**TABLE 2**
**Experimental Design and Materials and Methods in the Inhalation Studies of Cumene**

| 2-Week Studies | 3-Month Studies | 2-Year Studies |
|---|---|---|
| **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 twice daily on exposure days. | 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; for rats, clinical findings were recorded every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the study; for mice, clinical findings were recorded weekly through week 13, then every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the study; animals were weighed initially, weekly for the first 13 weeks, then every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the studies. |
| **Method of Sacrifice** | | |
| Carbon dioxide asphyxiation | Same as 2-week studies | Same as 2-week studies |
| **Necropsy** | | |
| Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus. | Necropsies were performed on core study animals. Organs weighed were 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 at the end of the study for hematology and clinical chemistry. Blood was collected from the retroorbital sinus of mice at the end of the study for hematology.<br>***Hematology:*** hematocrit; packed red cell volume; hemoglobin; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte counts and differentials.<br>***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids | None |

**TABLE 2**
**Experimental Design and Materials and Methods in the Inhalation Studies of Cumene**

| 2-Week Studies | 3-Month Studies | 2-Year Studies |
|---|---|---|
| **Histopathology** | | |
| Histopathology was performed on 0, 2,000, and 4,000 ppm rats and 0, 1,000, 2,000, and 4,000 ppm mice. In addition to gross lesions and tissue masses, the following tissues were examined: kidney, liver, lung, and nose. These tissues were examined to a no-effect level in the remaining exposure groups. | Complete histopathology was performed on 0 and 1,000 ppm core study rats and 0, 500 (females only), and 1,000 ppm mice. 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 and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with mainstem bronchus), lymph nodes (mandibular, mesenteric, bronchial, 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. In addition, the kidney of male rats in the remaining groups, the liver of 500 ppm male mice, and the forestomach of 250 ppm female mice were examined. | Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with bronchus), lymph nodes (mandibular, mesenteric, bronchial, 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 male animals in the 0, 250, 500, and 1,000 ppm groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from females exposed to 0, 125 (mice), 250, 500, or 1,000 (rats) ppm for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated. | None |
| **Renal Toxicity Study** | | |
| None | At the end of the study, concentrations of $\alpha$2u-globulin and soluble protein were measured in the right kidney of core study rats; the left kidneys were used for evaluation of hyaline droplets, assessment of cell proliferation indices, and histopathology. | 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 D4 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 exposed group with controls and a test for an overall exposure-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 exposed 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). Hemato-logy, clinical chemistry, renal toxicity, 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.

### 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 new 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 cumene 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 rat bone marrow and 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

Rats exposed to 4,000 ppm cumene died during or after the first exposure on day 1, and half (2/5 males and 3/5 females) of the rats exposed to 2,000 ppm died by day 4 (Table 3). Final mean body weights and body weight gains of 2,000 ppm rats were significantly less than those of the chamber controls. Rats exposed to 2,000 ppm that died early were severely ataxic and lethargic on the day of exposure and into the following morning. Rats exposed to 1,000 ppm and those that survived exposure to 2,000 ppm exhibited varying degrees of lethargy or ataxia, although the rats appeared to devel-op a tolerance, and the severity of clinical effects lessened by the end of week 1. Rats exposed to 500 ppm exhibited mild ataxia only after the initial exposure on day 1.

Significant increases in absolute and relative weights of the liver and relative weights of the kidney occurred in all exposed groups (Table G1). The absolute kidney weights of 250 and 1,000 ppm males and 250, 500,

**TABLE 3**
**Survival and Body Weights of Rats in the 2-Week Inhalation Study of Cumene**

| Concentration (ppm) | Survival[a] | Mean Body Weight[b] (g) | | | Final Weight Relative to Controls (%) |
|---|---|---|---|---|---|
| | | Initial | Final | Change | |
| **Male** | | | | | |
| 0 | 5/5 | 82 ± 2 | 146 ± 4 | 64 ± 4 | |
| 250 | 5/5 | 84 ± 2 | 149 ± 4 | 65 ± 3 | 103 |
| 500 | 5/5 | 83 ± 3 | 148 ± 7 | 66 ± 5 | 102 |
| 1,000 | 5/5 | 84 ± 2 | 148 ± 5 | 63 ± 3 | 101 |
| 2,000 | 3/5[c] | 85 ± 3 | 102 ± 3** | 15 ± 3** | 70 |
| 4,000 | 0/5[d] | 86 ± 2 | — | — | — |
| **Female** | | | | | |
| 0 | 5/5 | 79 ± 2 | 120 ± 3 | 41 ± 1 | |
| 250 | 5/5 | 78 ± 2 | 122 ± 3 | 43 ± 2 | 101 |
| 500 | 5/5 | 78 ± 2 | 125 ± 1 | 47 ± 2 | 104 |
| 1,000 | 5/5 | 80 ± 2 | 122 ± 3 | 43 ± 2 | 102 |
| 2,000 | 2/5[e] | 79 ± 3 | 93 ± 3** | 10 ± 2** | 78 |
| 4,000 | 0/5[d] | 79 ± 3 | — | — | — |

** Significantly different ($P \leq 0.01$) from the chamber control group by Dunnett's test
[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, 4
[d] Day of deaths: 1
[e] Days of death: 2, 3, 4

and 1,000 ppm females were significantly increased. Absolute and relative thymus weights were significantly decreased in the 2,000 ppm groups. No exposure-related gross lesions were observed.

Accumulation of minimal to mild hyaline droplets was observed in the renal tubular cortex of males exposed to 250 to 2,000 ppm, and the incidences were greatest in the 250 and 1,000 ppm groups [chamber controls, 0/5; 250 ppm, 3/5 (1.3); 500 ppm, 2/5 (1.5); 1,000 ppm, 3/5 (1.0); 2,000 ppm, 1/5 (1.0); 4,000 ppm, 0/5]. The lack of hyaline droplets in 2,000 ppm males that died on days 2 and 4 and 4,000 ppm males that died on day 1 was considered the result of the short period of exposure.

*Exposure Concentration Selection Rationale:* In male rats, increased incidences of hyaline droplet accumulation in the renal cortex were caused by cumene exposure. There was no evidence of other renal tubule epithelium damage. Based on the mortalities at 2,000 and 4,000 ppm, organ weight changes, and the lack of lesions in tissues examined, cumene exposure concentrations selected for the 3-month inhalation study in rats were 62.5, 125, 250, 500, and 1,000 ppm.

## 3-Month Study

All rats survived to the end of the study (Table 4). Final mean body weights and body weight gains of all exposed groups were similar to those of the chamber controls. Male rats exposed to 1,000 ppm exhibited ataxia for several days but recovered and appeared to develop a tolerance by day 3.

The hematology and clinical chemistry data for rats in the 3-month inhalation study of cumene are listed in Tables 5 and F1. The most consistent changes involved markers of hepatic injury/function. Serum total bile acid concentrations were significantly increased in essentially all exposed groups on days 3 and 23 of the study. The bile acid increases occurred in an exposure concentration-related manner (ranging from approximately 20% in the 62.5 ppm groups to approximately 65% in the 1,000 ppm groups). This effect was, however, transient and by study termination affected only the 500 and 1,000 ppm males. Markers of hepatocyte injury, alanine aminotransferase and sorbitol dehydrogenase activities, demonstrated an exposure concentration-related decrease in serum activity. Alanine aminotransferase appeared to be most affected, demonstrating some effect at all time points; the sorbitol dehydrogenase effect was limited to week 14. The magnitude of the effect intensified with time, and by study termination, the 250, 500, and 1,000 ppm male and female groups demonstrated decreases in serum activity of these two enzymes (up to an approximate 58% decrease in alanine aminotransferase activity in the 1,000 ppm males and females). Serum alkaline phosphatase activity (a marker of hepatobiliary function) also demonstrated minimal exposure concentration-related decreases; the decreases were significant in male rats exposed to 250 ppm or greater at week 14 and in 500 and 1,000 ppm females at all time points. No exposure-related lesions were observed in the livers of exposed rats. However, the relative liver weights increased with increasing exposure concentrations in essentially all male and female groups (Tables 6 and G2). The liver weight effects, coupled with the effects detected in the clinical chemistry, could suggest hepatic effects that altered liver function, resulting in

**Table 4**
**Survival and Body Weights of Rats in the 3-Month Inhalation Study of Cumene**

| Concentration (ppm) | Survival[a] | Mean Body Weight[b] (g) | | | Final Weight Relative to Controls (%) |
|---|---|---|---|---|---|
| | | Initial | Final | Change | |
| **Male** | | | | | |
| 0 | 10/10 | 89 ± 3 | 302 ± 8 | 213 ± 9 | |
| 62.5 | 10/10 | 91 ± 3 | 303 ± 6 | 212 ± 6 | 100 |
| 125 | 10/10 | 90 ± 3 | 313 ± 5 | 223 ± 6 | 104 |
| 250 | 10/10 | 91 ± 3 | 319 ± 4 | 228 ± 5 | 105 |
| 500 | 10/10 | 87 ± 3 | 303 ± 5 | 216 ± 6 | 100 |
| 1,000 | 10/10 | 89 ± 2 | 312 ± 5 | 223 ± 5 | 103 |
| **Female** | | | | | |
| 0 | 10/10 | 84 ± 2 | 190 ± 2 | 105 ± 3 | |
| 62.5 | 10/10 | 84 ± 2 | 184 ± 3 | 100 ± 3 | 97 |
| 125 | 10/10 | 81 ± 1 | 190 ± 4 | 109 ± 4 | 100 |
| 250 | 10/10 | 83 ± 2 | 187 ± 3 | 104 ± 3 | 99 |
| 500 | 10/10 | 85 ± 2 | 182 ± 3 | 97 ± 3 | 96 |
| 1,000 | 10/10 | 83 ± 2 | 184 ± 3 | 101 ± 3 | 97 |

[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 5**
**Selected Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene[a]**

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| **Male** | | | | | | |
| Albumin (g/dL) | | | | | | |
| Day 3 | 4.0 ± 0.1 | 3.8 ± 0.1 | 3.6 ± 0.1* | 3.6 ± 0.1** | 3.8 ± 0.1 | 3.8 ± 0.1 |
| Day 23 | 3.8 ± 0.1 | 3.7 ± 0.1 | 3.8 ± 0.1 | 3.8 ± 0.1 | 3.9 ± 0.1 | 3.9 ± 0.0 |
| Week 14 | 3.8 ± 0.1 | 3.9 ± 0.0 | 3.8 ± 0.0 | 3.9 ± 0.0 | 4.1 ± 0.1** | 4.2 ± 0.0** |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 3 | 64 ± 2 | 61 ± 1 | 61 ± 1 | 64 ± 2 | 60 ± 2 | 56 ± 1** |
| Day 23 | 44 ± 1 | 39 ± 1* | 39 ± 1** | 38 ± 1** | 35 ± 1** | 35 ± 0** |
| Week 14 | 113 ± 6 | 113 ± 11 | 110 ± 12 | 70 ± 4** | 61 ± 3** | 50 ± 2** |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 3 | 733 ± 14 | 759 ± 16 | 779 ± 14 | 794 ± 24 | 771 ± 24 | 674 ± 15 |
| Day 23 | 496 ± 15 | 490 ± 11 | 506 ± 15 | 485 ± 15 | 456 ± 27 | 470 ± 12 |
| Week 14 | 309 ± 7 | 294 ± 4 | 293 ± 10 | 283 ± 9* | 275 ± 7** | 250 ± 6** |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 3 | 13 ± 1 | 12 ± 1 | 12 ± 1 | 11 ± 1 | 12 ± 0 | 11 ± 1 |
| Day 23 | 13 ± 0 | 13 ± 0 | 13 ± 0 | 12 ± 1 | 12 ± 0 | 12 ± 0 |
| Week 14 | 26 ± 1 | 24 ± 2 | 22 ± 1 | 20 ± 1** | 17 ± 1** | 17 ± 1** |
| Bile acids (µmol/L) | | | | | | |
| Day 3 | 33.8 ± 1.1 | 39.9 ± 0.9** | 47.1 ± 0.7** | 50.9 ± 1.7** | 50.4 ± 2.0** | 55.6 ± 4.1** |
| Day 23 | 32.1 ± 2.9 | 33.2 ± 1.4 | 37.7 ± 1.4* | 41.1 ± 1.0** | 43.4 ± 1.7** | 44.2 ± 1.4** |
| Week 14 | 28.6 ± 1.7 | 31.2 ± 1.3 | 32.7 ± 1.5 | 30.9 ± 0.9 | 32.4 ± 0.9* | 35.9 ± 2.2** |
| **Female** | | | | | | |
| Albumin (g/dL) | | | | | | |
| Day 3 | 3.6 ± 0.1 | 3.6 ± 0.1 | 3.6 ± 0.1 | 3.5 ± 0.1 | 3.6 ± 0.0 | 3.7 ± 0.1 |
| Day 23 | 3.8 ± 0.0 | 3.8 ± 0.0 | 3.7 ± 0.0 | 3.9 ± 0.1 | 3.8 ± 0.1 | 3.8 ± 0.1 |
| Week 14 | 4.2 ± 0.1 | 4.0 ± 0.1 | 4.3 ± 0.1 | 4.2 ± 0.1 | 4.3 ± 0.1 | 4.6 ± 0.1** |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 3 | 50 ± 1 | 51 ± 2 | 48 ± 2 | 48 ± 2 | 47 ± 2 | 42 ± 2** |
| Day 23 | 37 ± 1 | 37 ± 1 | 34 ± 1 | 35 ± 1 | 34 ± 1 | 33 ± 1 |
| Week 14 | 80 ± 8 | 64 ± 6 | 63 ± 4 | 53 ± 4** | 53 ± 3** | 33 ± 1** |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 3 | 642 ± 18 | 636 ± 23 | 618 ± 19 | 608 ± 12 | 585 ± 15* | 474 ± 10** |
| Day 23 | 372 ± 6 | 388 ± 11 | 370 ± 8 | 362 ± 9 | 348 ± 7* | 319 ± 7** |
| Week 14 | 272 ± 9 | 275 ± 8 | 262 ± 12 | 267 ± 7 | 243 ± 7* | 207 ± 6** |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 3 | 13 ± 1 | 13 ± 0 | 13 ± 0 | 12 ± 0 | 12 ± 0 | 12 ± 0 |
| Day 23 | 12 ± 1 | 11 ± 0 | 11 ± 0 | 12 ± 0 | 11 ± 0 | 12 ± 0 |
| Week 14 | 22 ± 1 | 21 ± 1 | 19 ± 0 | 19 ± 1 | 18 ± 1** | 15 ± 1** |
| Bile acids (µmol/L) | | | | | | |
| Day 3 | 27.2 ± 1.3 | 32.6 ± 1.9* | 34.4 ± 1.8** | 38.5 ± 1.8** | 42.5 ± 0.9** | 38.0 ± 1.7** |
| Day 23 | 23.0 ± 0.7 | 27.6 ± 1.5** | 29.1 ± 0.9** | 31.5 ± 1.1** | 38.5 ± 2.9** | 38.4 ± 3.6** |
| Week 14 | 29.7 ± 2.6 | 24.3 ± 1.3 | 26.2 ± 1.6 | 32.1 ± 4.3 | 25.7 ± 2.3 | 23.8 ± 0.9 |

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

**TABLE 6**
**Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Inhalation Study of Cumene**[a]

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| **Male** | | | | | | |
| Necropsy body wt | 312 ± 8 | 313 ± 6 | 322 ± 5 | 331 ± 4 | 314 ± 5 | 323 ± 5 |
| R. Kidney | | | | | | |
| Absolute | 0.923 ± 0.024 | 0.980 ± 0.025 | 1.010 ± 0.031*[b] | 1.059 ± 0.021** | 1.070 ± 0.017** | 1.152 ± 0.023** |
| Relative | 2.962 ± 0.032 | 3.128 ± 0.051** | 3.131 ± 0.056**[b] | 3.194 ± 0.036** | 3.411 ± 0.045** | 3.561 ± 0.029** |
| Liver | | | | | | |
| Absolute | 9.518 ± 0.327 | 10.123 ± 0.267 | 10.260 ± 0.264 | 11.170 ± 0.302** | 11.589 ± 0.282** | 12.637 ± 0.288** |
| Relative | 30.481 ± 0.348 | 32.279 ± 0.278* | 31.792 ± 0.430* | 33.660 ± 0.558** | 36.895 ± 0.563** | 39.068 ± 0.549** |
| **Female** | | | | | | |
| Necropsy body wt | 195 ± 2 | 190 ± 3 | 194 ± 4 | 190 ± 3 | 185 ± 3 | 187 ± 4 |
| R. Kidney | | | | | | |
| Absolute | 0.637 ± 0.016 | 0.636 ± 0.010 | 0.649 ± 0.018 | 0.655 ± 0.017 | 0.645 ± 0.011 | 0.675 ± 0.011 |
| Relative | 3.263 ± 0.061 | 3.355 ± 0.049 | 3.322 ± 0.057 | 3.439 ± 0.057* | 3.486 ± 0.044** | 3.612 ± 0.040** |
| Liver | | | | | | |
| Absolute | 5.553 ± 0.130 | 5.669 ± 0.148 | 5.885 ± 0.204 | 5.959 ± 0.137 | 5.979 ± 0.133 | 6.923 ± 0.227** |
| Relative | 28.442 ± 0.389 | 29.858 ± 0.458 | 30.094 ± 0.634* | 31.289 ± 0.412** | 32.286 ± 0.386** | 36.958 ± 0.724** |

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

some perturbation in bile acid uptake or excretion and liver enzyme production/turnover or, possibly, inhibition. Serum albumin concentration (a nonspecific marker of hepatic function) was either unaffected or slightly (approximately 10%) increased in exposed males and females (Tables 5 and F1). The liver synthesizes albumin, and these results suggest that albumin synthesis was unaffected by exposure to cumene.

No exposure-related gross lesions were observed. Relative kidney and liver weights of all exposed groups of males, relative liver weights of 125 ppm or greater females, and relative kidney weights of 250 ppm or greater females were greater than those of the chamber controls (Tables 6 and G2). In addition, the absolute weights of the kidney were significantly increased in 125 ppm or greater males, and the absolute weights of the liver were increased in 250 ppm or greater males and 1,000 ppm females. There were no significant differences between exposed and chamber control males in reproductive tissue evaluations (Table H1). Exposed females differed significantly from the chamber control females in the relative length of time spent in estrus and proestrus (Table H2).

In cell proliferation analyses of the left kidney, the mean numbers of proximal tubule cells in the *S*-phase were significantly increased in the 500 and 1,000 ppm groups; however, the number of cells labeled and the labeling index were not significantly different from the chamber control group (Table 7). Concentrations of soluble protein and $\alpha$2u-globulin in the right kidney from male rats demonstrated increases in all exposed groups. Soluble protein levels were significantly increased in male rats exposed to 250 ppm or greater. Amounts and concentrations of $\alpha$2u-globulin were significantly increased in groups exposed to 125 ppm or greater. These findings are consistent with the hyaline droplet accumulations observed microscopically (Table 8).

The severities of hyaline droplet accumulation in the renal cortical tubules of exposed male rats were increased, and the incidences and severities of renal cortical tubule regeneration were generally slightly increased (Table 8). These changes were accompanied by significantly increased incidences of medullary granular casts in groups exposed to 250 ppm or greater. Hyaline droplet accumulation was observed in all male rats and was minimal to mild in the chamber control and 62.5 ppm groups, minimal to moderate at 125 ppm, and mild to moderate at 250 ppm or greater. In males, minimal tubular regeneration occurred in the chamber controls; minimal to mild lesions were observed in groups exposed to 62.5 or 125 ppm, and minimal to moderate tubular regeneration occurred in groups exposed to 250 ppm or greater. In males, minimal casts were observed in the 125 ppm group; minimal to moderate casts occurred in the 250 ppm group, and minimal to marked casts occurred in groups exposed to 500 or 1,000 ppm. In hematoxylin and eosin stained sections, hyaline droplets appeared as brightly eosinophilic globules of varying size in the renal cortical tubular epithelium. In Mallory-Heidenhain stained sections, hyaline droplets were magenta and were more easily visualized for severity evaluations. Tubular regeneration was characterized by tubules lined by more basophilic epithelium and having larger nuclei than the surrounding tubules. Affected tubules typically occurred in clusters. The granular casts were generally present at the corticomedullary junction and caused dilation of the tubular lumen with lightly eosinophilic granular acellular material.

*Exposure Concentration Selection Rationale:* In male rats, granular casts in the renal tubules of the medulla and increased severities of renal tubule regeneration and hyaline droplet accumulation in the cortex were caused by cumene exposure. The granular casts and renal tubule regeneration are indicative of some renal tubule epithelium damage. Based on the lack of mortalities and body weight effects, minimal organ weight changes, and the lack of lesions in other tissues, cumene exposure concentrations selected for the 2-year inhalation study in rats were 250, 500, and 1,000 ppm.

**TABLE 7**
**Renal Toxicity Data for Male Rats in the 3-Month Inhalation Study of Cumene[a]**

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Cells labeled | 60.60 ± 4.25 | 78.90 ± 5.60 | 63.80 ± 6.03 | 54.90 ± 4.50 | 43.70 ± 6.21 | 79.40 ± 6.65 |
| Cells counted | 2,095 ± 25 | 2,190 ± 28 | 2,100 ± 29 | 2,171 ± 31 | 2,268 ± 42 | 2,223 ± 45 |
| Labeling index (%)[b] | 2.894 ± 0.204 | 3.607 ± 0.266 | 3.051 ± 0.305 | 2.536 ± 0.219 | 1.944 ± 0.306 | 3.614 ± 0.346 |
| Soluble protein (mg/mL) | 21.22 ± 1.27 | 23.43 ± 0.51 | 23.93 ± 0.93[c] | 25.36 ± 0.69* | 25.51 ± 0.73** | 26.16 ± 1.09** |
| α2u-Globulin (nmol/g kidney) | 172.2 ± 22.3 | 328.1 ± 69.6 | 383.4 ± 46.3**[c] | 420.7 ± 50.1** | 363.2 ± 41.4** | 575.2 ± 74.8** |
| α2u-Globulin (ng/µg soluble protein) | 76.46 ± 9.24 | 130.98 ± 27.27 | 150.90 ± 19.23*[c] | 154.35 ± 16.98** | 133.01 ± 14.25** | 209.79 ± 31.34** |

* Significantly different (P≤0.05) from the chamber control group by Shirley's test
** P≤0.01
[a] Data are presented as mean ± standard error.
[b] Labeling index was calculated as the number of labeled cells divided by the total number of cells counted times 100. A minimum of 2,000 cells were counted.
[c] n=9

**TABLE 8**
**Incidences of Nonneoplastic Lesions in the Kidney of Male Rats in the 3-Month Inhalation Study of Cumene**

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| Number Examined Microscopically | 10 | 10 | 10 | 10 | 10 | 10 |
| Cortex Renal Tubule, Accumulation, Hyaline Droplet[a] | 10 (1.1)[b] | 10 (1.4) | 10 (1.9) | 10 (2.4) | 10 (3.0) | 10 (2.9) |
| Cortex Renal Tubule, Regeneration | 8 (1.0) | 6 (1.2) | 8 (1.5) | 10 (1.8) | 10 (2.1) | 10 (2.1) |
| Medulla, Casts Granular | 0 | 0 | 2 (1.0) | 8** (1.5) | 10** (2.5) | 9** (2.2) |

** Significantly different (P≤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

## 2-YEAR STUDY

### *Survival*

Estimates of 2-year survival probabilities for male and female rats are shown in Table 9 and in the Kaplan-Meier survival curves (Figure 2). Survival of all exposed groups of rats was similar to that of the chamber controls.

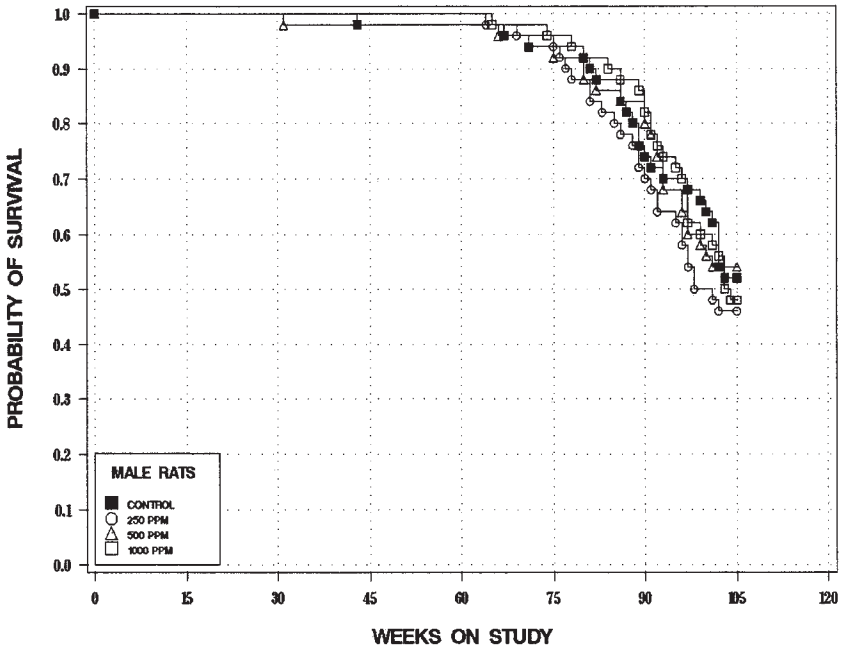
### *Body Weights and Clinical Findings*

Mean body weights of exposed groups of males were similar to those of the chamber controls throughout the study; mean body weights of 1,000 ppm females were slightly less than those of the chamber controls during the second year of the study but were similar to the chamber controls at the end of the study (Tables 10 and 11; Figure 3). There were no clinical findings related to exposure to cumene. Seizures occurred in a few exposed males (chamber control, 0/50; 250 ppm, 1/50; 500 ppm, 4/50; 1,000 ppm, 1/50) and in a few chamber control and exposed females (6/50, 8/50, 5/50, 5/50). The seizures were clonic and of short duration. They were most frequently observed and recorded during daily animal care activities. The first seizure episode was observed in a female rat at 32 weeks of exposure. No evidence of brain lesions was found to account for the cause or effect of the clonic seizures noted clinically in the animals. Similar, sporadic seizures have been observed in F344/N rats in six other NTP inhalation or dermal studies at

**TABLE 9**
**Survival of Rats in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Male** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Moribund | 20 | 24 | 21 | 24 |
| Natural deaths | 4 | 3 | 2 | 2 |
| Animals surviving to study termination | 26 | 23 | 27 | 24 |
| Percent probability of survival at end of study[a] | 52 | 46 | 54 | 48 |
| Mean survival (days)[b] | 676 | 665 | 671 | 684 |
| Survival analysis[c] | P=0.994N | P=0.511 | P=1.000N | P=0.946 |
| **Female** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Moribund | 23 | 18 | 17 | 15 |
| Natural deaths | 6 | 5 | 2 | 3 |
| Animals surviving to study termination | 21 | 27 | 31 | 32 |
| Percent probability of survival at end of study | 42 | 54 | 62 | 64 |
| Mean survival (days) | 673 | 677 | 685 | 684 |
| Survival analysis | P=0.061N | P=0.357N | P=0.109N | P=0.071N |

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] 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**.



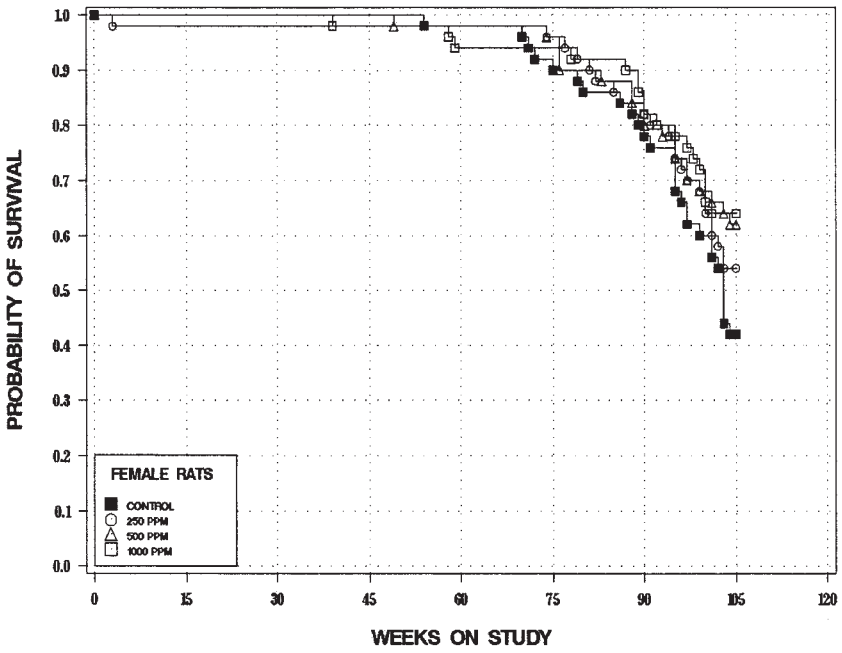




**FIGURE 2**
**Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Cumene by Inhalation for 2 Years**

**TABLE 10**
**Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Cumene**

| Days on Study | Chamber Control | | 250 ppm | | | 500 ppm | | | 1,000 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 | 107 | 50 | 106 | 99 | 50 | 106 | 99 | 50 | 105 | 97 | 50 |
| 9 | 145 | 50 | 144 | 99 | 50 | 142 | 98 | 50 | 137 | 94 | 50 |
| 16 | 176 | 50 | 175 | 99 | 50 | 174 | 99 | 50 | 169 | 96 | 50 |
| 23 | 202 | 50 | 204 | 101 | 50 | 203 | 101 | 50 | 198 | 98 | 50 |
| 30 | 224 | 50 | 226 | 101 | 50 | 227 | 101 | 50 | 221 | 98 | 50 |
| 37 | 245 | 50 | 247 | 101 | 50 | 248 | 101 | 50 | 243 | 99 | 50 |
| 44 | 260 | 50 | 261 | 100 | 50 | 264 | 101 | 50 | 258 | 99 | 50 |
| 51 | 275 | 50 | 275 | 100 | 50 | 278 | 101 | 50 | 272 | 99 | 50 |
| 58 | 288 | 50 | 289 | 100 | 50 | 292 | 101 | 50 | 287 | 100 | 50 |
| 65 | 300 | 50 | 302 | 100 | 50 | 305 | 101 | 50 | 301 | 100 | 50 |
| 72 | 311 | 50 | 311 | 100 | 50 | 316 | 102 | 50 | 310 | 100 | 50 |
| 79 | 321 | 50 | 321 | 100 | 50 | 326 | 102 | 50 | 321 | 100 | 50 |
| 86 | 328 | 50 | 328 | 100 | 50 | 336 | 103 | 50 | 330 | 101 | 50 |
| 114 | 363 | 50 | 360 | 99 | 50 | 368 | 101 | 50 | 362 | 100 | 50 |
| 142 | 391 | 50 | 385 | 99 | 50 | 394 | 101 | 50 | 388 | 99 | 50 |
| 170 | 415 | 50 | 411 | 99 | 50 | 419 | 101 | 50 | 408 | 98 | 50 |
| 198 | 432 | 50 | 427 | 99 | 50 | 434 | 101 | 50 | 423 | 98 | 50 |
| 226 | 446 | 50 | 442 | 99 | 50 | 449 | 101 | 49 | 441 | 99 | 50 |
| 254 | 461 | 50 | 455 | 99 | 50 | 464 | 101 | 49 | 456 | 99 | 50 |
| 282 | 473 | 50 | 467 | 99 | 50 | 476 | 101 | 49 | 465 | 98 | 50 |
| 310 | 480 | 49 | 474 | 99 | 50 | 485 | 101 | 49 | 473 | 99 | 50 |
| 338 | 490 | 49 | 482 | 98 | 50 | 490 | 100 | 49 | 484 | 99 | 50 |
| 366 | 496 | 49 | 490 | 99 | 50 | 499 | 100 | 49 | 492 | 99 | 50 |
| 394 | 507 | 49 | 500 | 99 | 50 | 508 | 100 | 49 | 500 | 99 | 50 |
| 422 | 513 | 49[a] | 509 | 99 | 50 | 517 | 101 | 49 | 504 | 98 | 50 |
| 450 | 519 | 49 | 514 | 99 | 49 | 518 | 100 | 49 | 504 | 97 | 50 |
| 478 | 523 | 48 | 510 | 98 | 49 | 520 | 100 | 48 | 511 | 98 | 49 |
| 506 | 531 | 47 | 523 | 99 | 48 | 527 | 99 | 47 | 517 | 97 | 49 |
| 534 | 534 | 47 | 521 | 98 | 46 | 529 | 99 | 46 | 515 | 97 | 48 |
| 562 | 536 | 46 | 528 | 99 | 44 | 534 | 100 | 44 | 523 | 98 | 46 |
| 591 | 540 | 44 | 534 | 99 | 40 | 532 | 99 | 43 | 524 | 97 | 45 |
| 618 | 540 | 40 | 529 | 98 | 38 | 537 | 99 | 42 | 517 | 96 | 44 |
| 646 | 539 | 36 | 534 | 99 | 32 | 534 | 99 | 36 | 524 | 97 | 38 |
| 660 | 541 | 35 | 524 | 97 | 32 | 535 | 99 | 34 | 517 | 96 | 37 |
| 674 | 532 | 35 | 525 | 99 | 29 | 534 | 100 | 32 | 514 | 96 | 35 |
| 688 | 529 | 34 | 541 | 102 | 25 | 529 | 100 | 30 | 515 | 97 | 31 |
| 702 | 527 | 32 | 533 | 101 | 25 | 527 | 100 | 28 | 509 | 97 | 30 |
| 716 | 531 | 27 | 536 | 101 | 23 | 527 | 99 | 27 | 500 | 94 | 28 |
| **Mean for weeks** | | | | | | | | | | | |
| 1-13 | 245 | | 245 | 100 | | 247 | 101 | | 242 | 99 | |
| 14-52 | 439 | | 434 | 99 | | 442 | 101 | | 433 | 99 | |
| 53-103 | 527 | | 522 | 99 | | 525 | 100 | | 512 | 97 | |

[a] Number of animals weighed was less than number of animals surviving.

**TABLE 11**
**Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of Cumene**

| Days on Study | Chamber Control | | 250 ppm | | | 500 ppm | | | 1,000 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 | 89 | 50 | 89 | 100 | 50 | 88 | 99 | 50 | 88 | 99 | 50 |
| 9 | 110 | 50 | 110 | 100 | 50 | 110 | 100 | 50 | 107 | 98 | 50 |
| 16 | 124 | 50 | 124 | 100 | 49 | 125 | 101 | 50 | 123 | 99 | 50 |
| 23 | 135 | 50 | 138 | 102 | 49 | 138 | 102 | 50 | 137 | 101 | 50 |
| 30 | 147 | 50 | 150 | 102 | 49 | 149 | 101 | 50 | 149 | 101 | 50 |
| 37 | 157 | 50 | 160 | 102 | 49 | 159 | 101 | 50 | 158 | 101 | 50 |
| 44 | 164 | 50 | 167 | 102 | 49 | 166 | 101 | 50 | 166 | 101 | 50 |
| 51 | 171 | 50 | 172 | 101 | 49 | 171 | 100 | 50 | 171 | 100 | 50 |
| 58 | 174 | 50 | 176 | 101 | 49 | 175 | 100 | 50 | 175 | 100 | 50 |
| 65 | 180 | 50 | 182 | 101 | 49 | 180 | 100 | 50 | 180 | 100 | 50 |
| 72 | 183 | 50 | 186 | 102 | 49 | 185 | 101 | 50 | 183 | 100 | 50 |
| 79 | 188 | 50 | 191 | 102 | 49 | 189 | 101 | 50 | 188 | 100 | 50 |
| 86 | 191 | 50 | 193 | 101 | 49 | 193 | 101 | 50 | 190 | 100 | 50 |
| 114 | 204 | 50 | 208 | 102 | 49 | 205 | 100 | 50 | 202 | 99 | 50 |
| 142 | 215 | 50 | 217 | 101 | 49 | 216 | 100 | 50 | 211 | 98 | 50 |
| 170 | 227 | 50 | 231 | 102 | 49 | 227 | 100 | 50 | 222 | 98 | 50 |
| 198 | 234 | 50 | 236 | 101 | 49 | 233 | 100 | 50 | 227 | 97 | 50 |
| 226 | 241 | 50 | 243 | 101 | 49 | 240 | 100 | 50 | 233 | 97 | 50 |
| 254 | 251 | 50 | 253 | 101 | 49 | 248 | 99 | 50 | 239 | 95 | 50 |
| 282 | 259 | 50 | 260 | 100 | 49[a] | 255 | 98 | 50 | 246 | 95 | 49 |
| 310 | 269 | 50 | 270 | 100 | 49 | 262 | 98 | 50 | 251 | 94 | 49 |
| 338 | 281 | 50 | 282 | 100 | 49 | 269 | 96 | 50 | 259 | 92 | 49 |
| 366 | 294 | 50 | 293 | 100 | 49 | 282 | 96 | 49 | 269 | 91 | 49 |
| 394 | 302 | 49 | 304 | 101 | 49 | 292 | 97 | 49 | 279 | 92 | 49 |
| 422 | 312 | 49 | 316 | 101 | 49 | 306 | 98 | 49 | 291 | 93 | 47 |
| 450 | 318 | 49 | 320 | 101 | 49 | 312 | 98 | 49 | 298 | 94 | 47 |
| 478 | 323 | 49 | 326 | 101 | 49 | 317 | 98 | 49 | 303 | 94 | 47 |
| 506 | 336 | 46 | 338 | 101 | 49 | 326 | 97 | 49 | 314 | 94 | 47 |
| 534 | 341 | 45 | 344 | 101 | 48 | 330 | 97 | 45 | 320 | 94 | 47 |
| 562 | 346 | 43 | 345 | 100 | 46 | 336 | 97 | 45 | 327 | 95 | 46 |
| 591 | 352 | 43 | 354 | 100 | 44 | 341 | 97 | 44 | 334 | 95 | 46 |
| 618 | 355 | 41 | 361 | 102 | 42 | 343 | 97 | 42 | 333 | 94 | 45 |
| 646 | 354 | 38 | 362 | 102 | 40 | 346 | 98 | 39 | 339 | 96 | 40 |
| 660 | 349 | 38 | 364 | 104 | 39 | 348 | 100 | 39 | 341 | 98 | 39 |
| 674 | 350 | 33 | 366 | 104 | 35 | 350 | 100 | 36 | 342 | 98 | 39 |
| 688 | 348 | 30 | 363 | 104 | 35 | 349 | 100 | 35 | 344 | 99 | 37 |
| 702 | 343 | 30 | 370 | 108 | 32 | 351 | 102 | 34 | 348 | 101 | 33 |
| 716 | 344 | 26 | 373 | 108 | 28 | 356 | 104 | 32 | 351 | 102 | 32 |
| **Mean for weeks** | | | | | | | | | | | |
| 1-13 | 155 | | 157 | 101 | | 156 | 101 | | 155 | 100 | |
| 14-52 | 242 | | 244 | 101 | | 239 | 99 | | 232 | 96 | |
| 53-103 | 335 | | 344 | 103 | | 330 | 99 | | 321 | 96 | |

[a] Number of animals weighed was less than number of animals surviving.

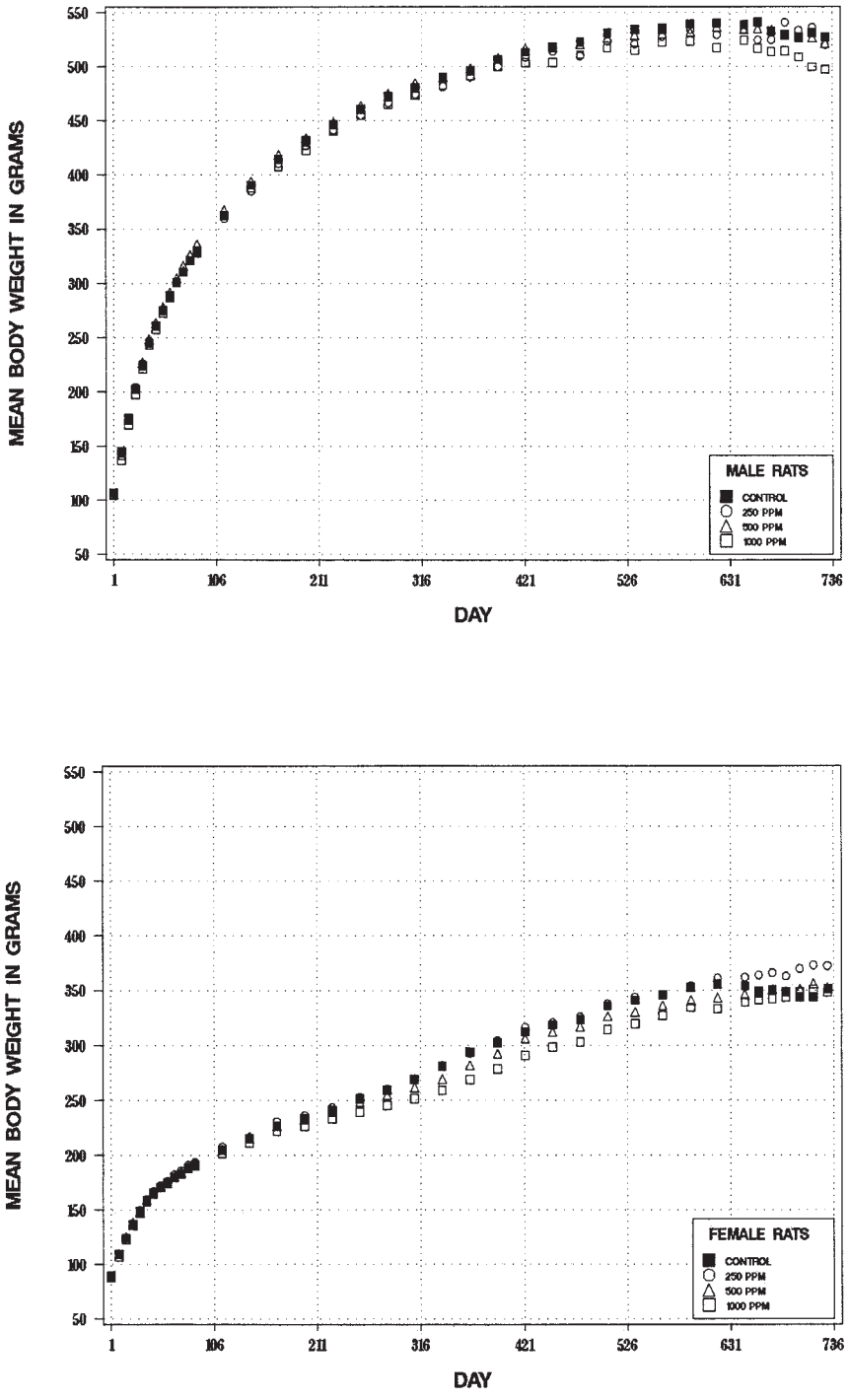



**FIGURE 3**
**Growth Curves for Male and Female Rats Exposed to Cumene by Inhalation for 2 Years**

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 animals 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 histopathologic 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.

### *Pathology and Statistical Analyses*

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the nose, kidney, and testis. 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:* Exposure to cumene resulted in increased incidences of adenoma of the respiratory epithelium, respiratory epithelium hyperplasia, olfactory epithelium basal cell hyperplasia, and goblet cell hyperplasia (Tables 12, A1, A2, A4, B1, B2, and B4). These nasal lesions were seen in all three nasal cavity levels that are routinely examined in NTP toxicity and carcinogenicity studies. Level I is excised immediately posterior to the upper incisor teeth; Level II is excised through the level of the incisive papilla anterior to the first palatial ridge, and Level III is excised through the middle of the second molar teeth. Levels I and II contain the naso- and maxilloturbinates that, along with the nasal passages (meatuses) and septum, are primarily lined by ciliated respiratory type epithelium. Level III encompasses the olfactory region of the nose, with ethmoid turbinates and meatuses lined entirely by specialized olfactory neuroepithelium (Boorman *et al.*, 1990).

Incidences of adenoma of the respiratory epithelium occurred with a positive trend in males and were significantly increased in all exposed groups of males and in 250 ppm females; the incidences in all exposed groups of rats exceeded the ranges for historical chamber controls in inhalation studies and historical controls (all routes) (Tables 12, A2, A3a, B2, and B3). The incidences of multiple adenomas in the respiratory epithelium of exposed groups of males increased with increasing exposure concentration, and the incidence in the 1,000 ppm group was significantly increased. Microscopically, the adenomas were rounded masses or papillary projections arising from the turbinates or the lateral wall and protruding into the nasal cavity at section Levels I and II (Plate 1). The adenomas consisted of cords or clusters of basophilic cuboidal epithelial cells that often formed acinar patterns (Plates 2 and 3).

Incidences of hyperplasia of basal cells in the olfactory epithelium were significantly increased in all exposed groups (Tables 12, A4, and B4). Basal cell hyperplasia was characterized by increased numbers of basal cells crowded along the basement membrane of the olfactory epithelium, most often affecting cells lining the nasal septum (Plates 4 and 5). In more severe lesions, affected basal cells infiltrated the overlying epithelium and occasionally formed small rosettes within the olfactory epithelium.

The incidences of hyperplasia of the respiratory epithelium were significantly increased in all exposed groups of males, and the incidence of this lesion was significantly increased in 1,000 ppm females (Tables 12, A4, and B4). Hyperplasia of the respiratory epithelium and adenoma form a morphologic continuum. Hyperplastic lesions consisted of minimal to marked increases in populations of homogeneous respiratory epithelial cells (Plate 6) with less distinct acinar formation than adenomas.

The incidence of goblet cell hyperplasia was significantly increased in 250 ppm males (Tables 12 and A4). Goblet cell hyperplasia was characterized by minimal to moderate increases in the number of mucous cells in respiratory epithelium. Hyperplasia was often accompanied by enlargement or hypertrophy of the mucous cells. The hyperplasia resulted in thickening of the respiratory epithelium and an undulating surface. The affected cells were taller, with many large cells containing abundant amounts of mucin. The nuclei appeared more numerous. Similar goblet cell hyperplasia with hypertrophy was observed in the olfactory epithelium of a 500 ppm male rat (Plate 7).

*Kidney:* The incidences of renal tubule adenoma were increased in all exposed groups of males compared to the chamber control group and exceeded the historical range for chamber controls in inhalation studies (Tables 13, A2, and A3b). The incidences of renal tubule carcinoma were increased in 500 and 1,000 ppm males and exceeded the historical chamber control range; one 500 ppm male rat had a bilateral renal tubule carcinoma. The incidence of renal tubule adenoma or carcinoma (combined) was significantly increased in 500 ppm males, and the incidences of these combined lesions in all exposed groups of males exceeded the range for historical chamber controls.

Six of the renal tubule adenomas were large enough to be observed grossly at necropsy and were described as nodules or lesions varying in size from 1 to 2 mm in

**TABLE 12**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Nose in Rats in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Male** | | | | |
| Number Examined Microscopically | 50 | 50 | 49 | 50 |
| Olfactory Epithelium, Hyperplasia, Basal Cell[a] | 0 | 19** (1.1)[b] | 27** (1.0) | 26** (1.0) |
| Respiratory Epithelium, Hyperplasia | 0 | 15** (2.0) | 16** (2.9) | 23** (2.7) |
| Goblet Cell, Hyperplasia | 3 (1.7) | 11* (2.3) | 7 (2.3) | 5 (2.0) |
| Glands, Respiratory Epithelium, Adenoma | 0 | 0 | 1 | 0 |
| Respiratory Epithelium, Adenoma, Multiple | 0 | 1 | 2 | 6* |
| Respiratory Epithelium, Adenoma (includes multiple and all sites)[c] | | | | |
| Overall rate[d] | 0/50 (0%) | 7/50 (14%) | 18/49 (37%) | 10/50 (20%) |
| Adjusted rate[e] | 0.0% | 17.6% | 43.2% | 23.3% |
| Terminal rate[f] | 0/26 (0%) | 5/23 (22%) | 13/27 (48%) | 7/24 (29%) |
| First incidence (days) | —[g] | 639 | 638 | 674 |
| Poly-3 test[h] | P=0.004 | P=0.006 | P<0.001 | P<0.001 |
| **Female** | | | | |
| Number Examined Microscopically | 50 | 48 | 50 | 50 |
| Olfactory Epithelium, Hyperplasia, Basal Cell | 0 | 14** (1.0) | 25** (1.0) | 31** (1.1) |
| Respiratory Epithelium, Hyperplasia | 0 | 0 | 4 (3.0) | 6* (2.3) |
| Respiratory Epithelium, Adenoma[i] | | | | |
| Overall rate | 0/50 (0%) | 5/48 (10%) | 4/50 (8%) | 3/50 (6%) |
| Adjusted rate | 0.0% | 12.2% | 9.3% | 6.9% |
| Terminal rate | 0/21 (0%) | 5/27 (19%) | 4/31 (13%) | 2/32 (6%) |
| First incidence (days) | — | 730 (T) | 730 (T) | 638 |
| Poly-3 test | P=0.320 | P=0.030 | P=0.066 | P=0.130 |

(T) Terminal sacrifice
* 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 control groups (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 comparison 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 inhalation studies: 0/496; all routes: 0/1,343

**TABLE 13**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Male** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Renal Tubule, Hyperplasia[a] | 0 | 3 (3.3)[b] | 8** (2.6) | 6* (2.2) |
| Papilla, Mineralization | 5 (1.0) | 35** (1.7) | 44** (2.1) | 41** (2.1) |
| Pelvis, Transitional Epithelium, Hyperplasia | 3 (1.7) | 5 (1.8) | 14** (2.4) | 15** (2.0) |
| Nephropathy | 47 (2.3) | 47 (2.6) | 47 (2.9) | 50 (2.7) |
| Renal Tubule, Adenoma[c] | | | | |
| Overall rate[d] | 1/50 (2%) | 4/50 (8%) | 5/50 (10%) | 4/50 (8%) |
| Adjusted rate[e] | 2.4% | 10.0% | 12.1% | 9.3% |
| Terminal rate[f] | 1/26 (4%) | 1/23 (4%) | 2/27 (7%) | 2/24 (8%) |
| First incidence (days) | 729 (T) | 665 | 679 | 635 |
| Poly-3 test[g] | P=0.219 | P=0.165 | P=0.099 | P=0.187 |
| Renal Tubule, Carcinoma, Bilateral | 0 | 0 | 1 | 0 |
| Renal Tubule, Carcinoma (includes bilateral)[h] | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 3/50 (6%) | 3/50 (6%) |
| Adjusted rate | 2.4% | 2.5% | 7.3% | 7.0% |
| Terminal rate | 1/26 (4%) | 1/23 (4%) | 2/27 (7%) | 2/24 (8%) |
| First incidence (days) | 729 (T) | 729 (T) | 639 | 618 |
| Poly-3 test | P=0.180 | P=0.749 | P=0.302 | P=0.314 |
| Renal Tubule, Adenoma or Carcinoma[i] | | | | |
| Overall rate | 2/50 (4%) | 5/50 (10%) | 8/50 (16%) | 7/50 (14%) |
| Adjusted rate | 4.8% | 12.5% | 19.2% | 16.2% |
| Terminal rate | 2/26 (8%) | 2/23 (9%) | 4/27 (15%) | 4/24 (17%) |
| First incidence (days) | 729 (T) | 665 | 639 | 618 |
| Poly-3 test | P=0.087 | P=0.198 | P=0.044 | P=0.087 |
| Renal Tubule, Lipoma[j] | 1 | 0 | 0 | 1 |
| **Female** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Nephropathy | 38 (1.4) | 37 (1.5) | 41 (1.9) | 44 (1.9) |

(T) Terminal sacrifice
* 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 control groups (mean ± standard deviation): 4/449 (0.9% ± 1.0%), range 0%-2%; all routes: 8/1,436 (0.6% ± 0.8%), range 0%-2%
[d] Number of animals with neoplasm per number of animals with kidney examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] 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 comparison between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence for inhalation studies: 2/449 (0.4% ± 0.9%), range 0%-2%; all routes: 2/1,436 (0.1% ± 0.5%), range 0%-2%
[i] Historical incidence for inhalation studies: 6/449 (1.3% ± 1.4%), range 0%-4%; all routes: 10/1,436 (0.7% ± 1.0%), range 0%-4%
[j] Historical incidence for inhalation studies: 1/449 (0.2% ± 0.7%), range 0%-2%; all routes: 2/1,436 (0.1% ± 0.5%), range 0%-2%

diameter. Microscopically, renal tubule adenomas were typically well circumscribed, discrete, rounded or oval masses greater than five times the diameter of a normal renal tubule, composed of solid aggregates, small nests or tubule-like structures of neoplastic epithelial cells without obvious lumens. The neoplastic cells were round to polygonal in shape with pale-staining, foamy cytoplasm. Larger adenomas had areas of cells with central vacuolation. Seven of the renal tubule cell carcinomas were observed at necropsy and were described as masses or nodules varying in size from 2 mm to 1.5 cm in diameter or as a dilated, thickened pelvis. Microscopically, renal tubule carcinomas were large, less discrete masses of neoplastic cells that were locally invasive and composed of a mixture of round cells with large vesicular nuclei and abundant, pale eosinophilic cytoplasm forming sheets and large nests resembling tubules. Some carcinomas had small numbers of vacuolated, signet ring, neoplastic cells. A few carcinomas had areas of necrosis or tubular structures with necrotic centers surrounded by a thin fibrous stroma. Cellular atypia and pleomorphism were usually present.

One chamber control and one 1,000 ppm male had a lipoma, a benign tumor (Tables 13 and A1). The lipoma in the chamber control male occupied the majority of the renal medulla and was composed of sheets of vacuolated cells with a few residual renal tubules and cystic spaces within the tumor. The lipoma in the 1,000 ppm male also was primarily composed of vacuolated cells but was located in the cortex. These lipomas are infrequent tumors in F344/N rats and unrelated to exposure. The tumor in the chamber control male is the only lipoma in the current historical control data for inhalation studies, and only one other lipoma is included for all routes of exposure combined (Tables 13 and A3b).

Incidences of renal tubule hyperplasia were increased in all exposed groups of male rats and were significantly increased in the 500 and 1,000 ppm groups (Tables 13 and A4). Microscopically, renal tubular cell hyperplasia consisted of mild to marked foci having single to multiple cortical tubules composed of multiple layers of epithelial cells with pale-staining, foamy cytoplasm that partially filled the lumen and enlarged the tubules. The epithelial cells were variably enlarged with distinct cell borders, expanded eosinophilic cytoplasm, variable nuclear size, and multiple, enlarged nucleoli. Affected tubules were generally, but not always, larger than normal but less than five times the diameter of a normal renal tubule. Renal tubule hyperplasia, as defined in the current study, was distinguished from regenerative epithelial changes commonly seen as a part of nephropathy and was considered a preneoplastic lesion. Renal tubule hyperplasia, adenoma, and carcinoma are part of a morphologic continuum.

The incidences of mineralization of the renal papilla were significantly increased in all exposed groups of males (Tables 13 and A4). Microscopically, the mineralization consisted of elongated profiles of dark basophilic or black granular material within the lumens of the tubules. This linear pattern is consistent with mineralization associated with $\alpha$2u-globulin nephropathy.

Significantly increased incidences of minimal to marked hyperplasia of the transitional epithelium of the renal pelvis occurred in 500 and 1,000 ppm males (Tables 13 and A4). Hyperplasia of the transitional epithelium was characterized by increased numbers of cells in the epithelium lining the pelvis, often with formation of papillary projections. These are common findings in rats and often increase with increased severities of nephropathy.

Although the incidences of minimal to marked nephropathy in exposed groups of males and females were not significantly different from those in the chamber controls, the severity of nephropathy generally increased with increasing exposure concentration (Tables 13, A4, and B4).

*Testis:* The incidence of interstitial cell adenoma, including bilateral, in 1,000 ppm male rats was significantly increased compared to the chamber control group, and there was a positive trend in the incidences among all exposed groups (Tables 14 and A2). The incidence in the 1,000 ppm group exceeded the range for historical chamber controls in inhalation studies but was within the range for historical controls (all routes) (Tables 14 and A3c). There were increased incidences of bilateral interstitial cell adenoma in all exposed groups (Tables 14 and A1).

Interstitial cell hyperplasia and adenoma are common proliferative lesions in F344/N rats and will develop in nearly all male rats of this strain that are allowed to complete their natural life span. Interstitial cell adenomas begin as nodular aggregates of hyperplasia having characteristically large polyhedral cells with abundant finely

**TABLE 14**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Testis in Male Rats in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Interstitial Cell, Hyperplasia[a] | 12 (1.9)[b] | 18 (2.1) | 19 (2.2) | 9 (3.0) |
| Bilateral Interstitial Cell, Hyperplasia | 0 | 0 | 0 | 1 (2.0) |
| Interstitial Cell, Adenoma | 18 | 14 | 13 | 9 |
| Bilateral, Interstitial Cell, Adenoma | 18 | 24 | 27 | 37 |
| Interstitial Cell Adenoma (includes bilateral)[c] | | | | |
| Overall rate[d] | 36/50 (72%) | 38/50 (76%) | 40/50 (80%) | 46/50 (92%) |
| Adjusted rate[e] | 80.0% | 84.6% | 85.7% | 96.1% |
| Terminal rate[f] | 24/26 (92%) | 22/23 (96%) | 25/27 (93%) | 24/24 (100%) |
| First incidence (days) | 558 | 536 | 460 | 541 |
| Poly-3 test[g] | P=0.006 | P=0.370 | P=0.311 | P=0.007 |

[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 control groups (mean ± standard deviation): 345/449 (76.8% ± 5.9%), range 66%-84%; all routes: 1,242/1,449 (85.5% ± 8.7%), range 66%-98%
[d] Number of animals with neoplasm per number of animals with testis examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] 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 comparison between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

vacuolated foamy or granular eosinophilic cytoplasm. They have central nuclei with a prominent nucleolus and marginated chromatin. Less commonly, areas of hyperplasia have small spindle cells with dark basophilic nuclei, scant cytoplasm, and indistinct cell boundaries. The distinction between hyperplasia and adenoma can be unclear because interstitial cells can form a continuous morphologic spectrum from small foci of normal appearing cells to large masses extending between and causing compression of the surrounding seminiferous tubules with eventual replacement of the entire testis. An aggregate smaller than a seminiferous tubule is focal hyperplasia. Masses of interstitial cells with a diameter equal in size to or larger than a seminiferous tubule with some compression of adjacent tubules are considered adenomas. As a result of the frequent presence of both lesions, any testis in which an adenoma, a neoplastic change, was present along with hyperplasia of the interstitial cells, only the adenoma was diagnosed. However, when one testis had an adenoma and the contralateral testis had foci of hyperplasia, both diagnoses were recorded.

Incidences of interstitial cell hyperplasia were increased in 250 and 500 ppm males, and the severity was increased in 1,000 ppm males (Tables 14 and A4). Because of the exposure-related increase in incidences of bilateral interstitial cell adenoma, the incidences of interstitial cell hyperplasia are not well reflected in this data. It is uncertain how the increased incidences of interstitial cell adenoma and hyperplasia were related to cumene exposure.

# MICE

## 2-WEEK STUDY

All mice exposed to 4,000 ppm died on day 1; all mice exposed to 2,000 ppm died by the morning of day 2, and four female mice exposed to 1,000 ppm died by day 4 (Table 15). There were no exposure-related changes in mean body weights among survivors in either sex. Mice exposed to 2,000 ppm were severely lethargic after the first exposure. The four female mice exposed to 1,000 ppm that died early exhibited signs of lethargy and ataxia. Ataxia was most apparent during the first few days of exposure in week 1, and the severity of lethargy and ataxia was greater in females.

Compared to the chamber control groups, absolute and relative liver weights were increased in all groups of surviving male mice and the 250 and 500 ppm females (Table G3). Absolute and relative kidney weights were generally increased in both sexes of surviving mice compared to the chamber control groups; the increases were significant in 250 ppm males. No microscopic changes were present in either sex that would account for the increased liver or kidney weights or for the early deaths. Thymus weights were decreased in 1,000 ppm males.

*Exposure Concentration Selection Rationale:* Based on the mortalities at 2,000 and 4,000 ppm and signs of central nervous system effects, cumene exposure concentrations selected for the 3-month inhalation study in mice were 62.5, 125, 250, 500, and 1,000 ppm.

**TABLE 15**
**Survival and Body Weights of Mice in the 2-Week Inhalation Study of Cumene**

| Concentration (ppm) | Survival[a] | Mean Body Weight[b] (g) | | | Final Weight Relative to Controls (%) |
|---|---|---|---|---|---|
| | | Initial | Final | Change | |
| **Male** | | | | | |
| 0 | 5/5 | 21.9 ± 0.3 | 27.0 ± 0.5 | 5.2 ± 0.4 | |
| 250 | 5/5 | 22.6 ± 0.8 | 27.6 ± 0.6 | 5.0 ± 0.7 | 102 |
| 500 | 5/5 | 23.1 ± 0.8 | 26.7 ± 0.4 | 3.6 ± 0.6 | 99 |
| 1,000 | 5/5 | 23.1 ± 0.5 | 27.0 ± 0.9 | 3.9 ± 0.4 | 100 |
| 2,000 | 0/5[c] | 23.0 ± 0.5 | — | — | — |
| 4,000 | 0/5[d] | 22.7 ± 0.6 | — | — | — |
| **Female** | | | | | |
| 0 | 5/5 | 18.6 ± 0.4 | 22.5 ± 0.3 | 3.9 ± 0.2 | |
| 250 | 5/5 | 19.2 ± 0.6 | 22.6 ± 0.7 | 3.5 ± 0.4 | 100 |
| 500 | 5/5 | 19.3 ± 0.2 | 23.3 ± 0.6 | 4.0 ± 0.8 | 103 |
| 1,000 | 1/5[e] | 18.9 ± 0.4 | 23.8 | 3.6 | 106 |
| 2,000 | 0/5[c] | 18.9 ± 0.4 | — | — | — |
| 4,000 | 0/5[d] | 18.7 ± 0.7 | — | — | — |

[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. Differences from the chamber control group are not significant by Dunnett's test.
[c] Day of deaths: 2
[d] Day of deaths: 1
[e] Days of deaths: 3, 3, 3, 4

## 3-MONTH STUDY

With the exception of eight 1,000 ppm females, all mice survived to the end of the study (Table 16). Final mean body weights and body weight gains of males exposed to 250 ppm or greater were generally less than those of the chamber controls. Ataxia was observed for several days in 1,000 ppm males, but the animals recovered and appeared to develop a tolerance by day 3. The eight 1,000 ppm female mice that died during the first week of the study exhibited clinical signs of acute toxicity, including lethargy or ataxia.

There were no toxicologically relevant changes in the hematology variables for mice (Table F2).

Significant increases in absolute liver weights occurred in mice exposed to 500 or 1,000 ppm, and significant increases in relative liver weights occurred in groups exposed to 125 ppm or greater (Tables 17 and G4). Although the weight of the cauda epididymis and the spermatid count were significantly decreased in 1,000 ppm males, there were no other significant differences between exposed and chamber control groups in reproductive tissue evaluations in males or vaginal cytology parameters in females (Tables H3 and H4). No exposure-related gross lesions were observed.

Incidences of minimal to mild liver necrosis were significantly increased in male mice exposed to 1,000 ppm (Table 18). Incidences of focal chronic inflammation were significantly increased in females exposed to 62.5, 125, 250, or 500 ppm. Necrosis varied from tiny foci of lymphocytes with a few deeply eosinophilic individual

**TABLE 16**
**Survival and Body Weights of Mice in the 3-Month Inhalation Study of Cumene**

| Concentration (ppm) | Survival[a] | Mean Body Weight[b] (g) | | | Final Weight Relative to Controls (%) |
|---|---|---|---|---|---|
| | | Initial | Final | Change | |
| **Male** | | | | | |
| 0 | 10/10 | 23.4 ± 0.3 | 37.5 ± 0.6 | 14.1 ± 0.7 | |
| 62.5 | 10/10 | 23.1 ± 0.3 | 36.1 ± 0.8 | 13.0 ± 0.7 | 96 |
| 125 | 10/10 | 23.2 ± 0.5 | 35.8 ± 0.8 | 12.6 ± 0.8 | 96 |
| 250 | 10/10 | 22.9 ± 0.2 | 35.3 ± 0.7* | 12.4 ± 0.6 | 94 |
| 500 | 10/10 | 22.9 ± 0.3 | 34.5 ± 0.8** | 11.6 ± 0.8* | 92 |
| 1,000 | 10/10 | 23.2 ± 0.3 | 34.1 ± 0.6** | 10.9 ± 0.7** | 91 |
| **Female** | | | | | |
| 0 | 10/10 | 19.3 ± 0.3 | 30.6 ± 1.0 | 11.3 ± 0.8 | |
| 62.5 | 10/10 | 19.0 ± 0.2 | 29.7 ± 1.0 | 10.7 ± 0.9 | 97 |
| 125 | 10/10 | 19.5 ± 0.2 | 29.6 ± 0.8 | 10.1 ± 0.8 | 97 |
| 250 | 10/10 | 19.3 ± 0.3 | 29.6 ± 0.8 | 10.3 ± 0.9 | 97 |
| 500 | 10/10 | 19.3 ± 0.3 | 28.3 ± 0.5 | 9.0 ± 0.3 | 92 |
| 1,000 | 2/10[c] | 19.1 ± 0.3 | 29.2 ± 0.4 | 8.9 ± 1.1 | 95 |

* Significantly different (P≤0.05) from the chamber control group by Williams' test
** P≤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. Subsequent calculations are based on animals surviving to the end of the study.
[c] Week of deaths: 1

**TABLE 17**
**Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Inhalation Study of Cumene[a]**

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 38.3 ± 0.7 | 37.7 ± 0.9 | 37.0 ± 0.8 | 36.1 ± 0.8 | 35.8 ± 0.9* | 34.7 ± 0.6** |
| Liver | | | | | | |
| Absolute | 1.531 ± 0.027 | 1.601 ± 0.049 | 1.607 ± 0.041 | 1.591 ± 0.048 | 1.705 ± 0.048* | 1.913 ± 0.070** |
| Relative | 40.048 ± 0.559 | 42.490 ± 0.894 | 43.485 ± 0.551* | 44.052 ± 0.746** | 47.668 ± 0.795** | 55.103 ± 1.501** |
| **Female** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 2 |
| Necropsy body wt | 32.4 ± 1.1 | 31.0 ± 1.2 | 31.4 ± 1.1 | 31.5 ± 1.1 | 29.8 ± 0.7 | 30.8 ± 1.3 |
| Liver | | | | | | |
| Absolute | 1.453 ± 0.037 | 1.430 ± 0.047 | 1.495 ± 0.053 | 1.552 ± 0.045 | 1.593 ± 0.042* | 1.910 ± 0.110** |
| Relative | 45.016 ± 1.043 | 46.200 ± 0.778 | 47.622 ± 0.576* | 49.380 ± 0.521** | 53.510 ± 0.663** | 62.071 ± 1.054** |

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' 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).

necrotic hepatocytes to foci having coagulative or liquefactive necrosis with scattered neutrophils and lymphocytes involving hepatocytes and adjacent liver tissues. Chronic inflammation consisted of lesions where the predominant features were clusters of lymphocytes and a few neutrophils. Liver necrosis and focal chronic inflammation in male and female mice were considered to be parts of a pathologic continuum.

Sporadic incidences of minimal to mild squamous epithelial hyperplasia and mild inflammation of the mucosa occurred in the forestomach of exposed groups of female mice (Table 18). Squamous epithelial hyperplasia consisted of minimal focal to diffuse thickening of the squamous epithelium. The affected epithelium was five to six cell layers thick as compared to the three to four cell layers for normal squamous epithelium. Acute and chronic inflammation of the forestomach was associated with hyperplasia in 500 and 1,000 ppm female mice. This inflammation was minimal, was confined to the lamina propria, and consisted of a mixture of inflammatory cells, primarily neutrophils and macrophages. The forestomach lesions had low incidences and were of uncertain relationship to cumene exposure.

Marked necrosis of the thymus was observed in the eight female mice exposed to 1,000 ppm cumene that died during the first week of the study (Table 18). The changes in the thymus were characterized by thymic lymphocytes with small, shrunken, dense nuclei. In multifocal areas, nuclei of the lymphocytes were fragmented or disintegrated. The thymic necrosis was considered to be a nonspecific terminal event associated with glucocorticoid release and not the cause of death; the cause of death was not explained.

**TABLE 18**
**Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Inhalation Study of Cumene**

| | Chamber Control | | 62.5 ppm | | 125 ppm | | 250 ppm | | 500 ppm | | 1,000 ppm | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | | | | | | |
| Liver[a] | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| Inflammation, Chronic Focal[b] | 4 | (1.0)[c] | 3 | (1.0) | 2 | (1.0) | 3 | (1.0) | 5 | (1.0) | 4 | (1.0) |
| Necrosis | 0 | | 1 | (1.0) | 1 | (2.0) | 1 | (1.0) | 1 | (1.0) | 5* | (1.2) |
| **Female** | | | | | | | | | | | | |
| Liver | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| Inflammation, Chronic Focal | 1 | (1.0) | 10** | (1.0) | 10** | (1.0) | 9** | (1.0) | 7** | (1.0) | 2 | (1.0) |
| Necrosis | 4 | (1.3) | 0 | | 0 | | 0 | | 2 | (1.5) | 0 | |
| Forestomach | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| Hyperplasia, Squamous | 0 | | 1 | (1.0) | 0 | | 0 | | 2 | (2.0) | 1 | (1.0) |
| Inflammation, Acute | 0 | | 0 | | 0 | | 0 | | 0 | | 1 | (1.0) |
| Inflammation, Chronic Active | 0 | | 0 | | 0 | | 2 | (1.0) | 2 | (1.0) | 0 | |
| Thymus | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| Necrosis | 0 | | 0 | | 0 | | 0 | | 0 | | 8** | (4.0) |

* 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 tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

*Exposure Concentration Selection Rationale:* Based on slight decreases in body weights in males and minimal effects on organ weights and incidences of lesions, cumene exposure concentrations selected for the 2-year inhalation study in male mice were 250, 500, and 1,000 ppm. Due to the lower survival rate for 1,000 ppm females, incidences of thymic necrosis at 1,000 ppm, and incidences of liver and forestomach lesions, cumene exposure concentrations selected for the 2-year inhalation study in female mice were 125, 250, and 500 ppm.

## 2-YEAR STUDY

### *Survival*

Estimates of 2-year survival probabilities for male and female mice are shown in Table 19 and in the Kaplan-Meier survival curves (Figure 4). An exposure concentration-related decrease in survival occurred in male mice, and the survival of 1,000 ppm males was significantly less than that of the chamber controls. Survival of exposed groups of female mice was similar to that of the chamber controls.

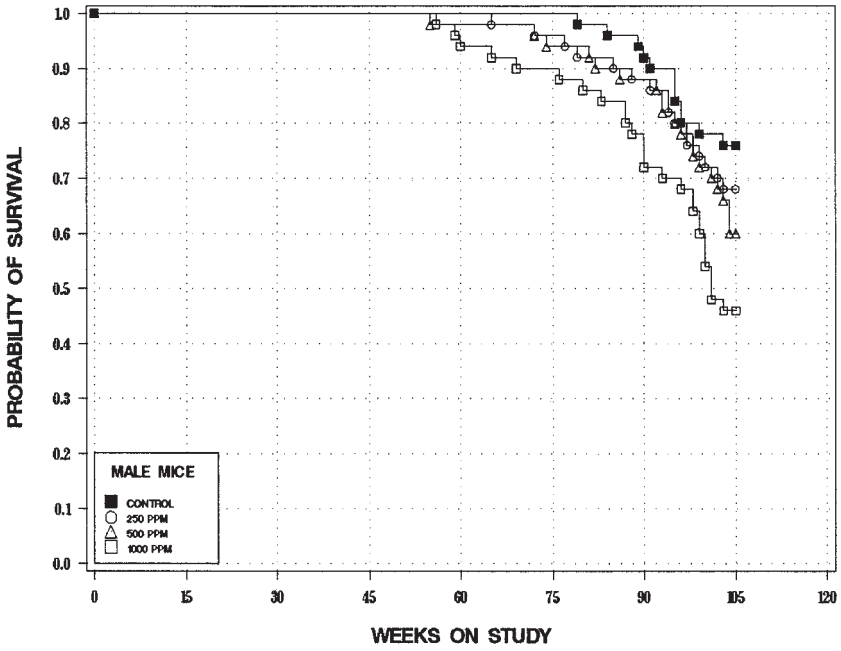
### *Body Weights and Clinical Findings*

Mean body weights of 1,000 ppm males were generally less than those of the chamber controls after week 8 of the study, and those of 500 ppm females were less from week 28 until week 76 of the study (Figure 5; Tables 20 and 21). There were no clinical findings related to exposure to cumene; however, thinness and abnormal breathing were observed somewhat more frequently in 1,000 ppm males and 500 ppm females late in the study.

**TABLE 19**
**Survival of Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Male** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Moribund | 7 | 9 | 13 | 20 |
| Natural deaths | 5 | 7 | 7 | 7 |
| Animals surviving to study termination | 38 | 34 | 30 | 23 |
| Percent probability of survival at end of study[a] | 76 | 68 | 60 | 46 |
| Mean survival (days)[b] | 709 | 696 | 693 | 665 |
| Survival analysis[c] | P=0.001 | P=0.475 | P=0.150 | P=0.004 |
| | **Chamber Control** | **125 ppm** | **250 ppm** | **500 ppm** |
| **Female** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Moribund | 8 | 10 | 8 | 12 |
| Natural deaths | 5 | 4 | 3 | 3 |
| Animals surviving to study termination | 37 | 36 | 39 | 35[d] |
| Percent probability of survival at end of study | 74 | 72 | 78 | 70 |
| Mean survival (days) | 685 | 698 | 712 | 714 |
| Survival analysis | P=0.996 | P=1.000 | P=0.728N | P=0.982 |

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] 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**.
[d] Includes one animal that died during the last week of the study



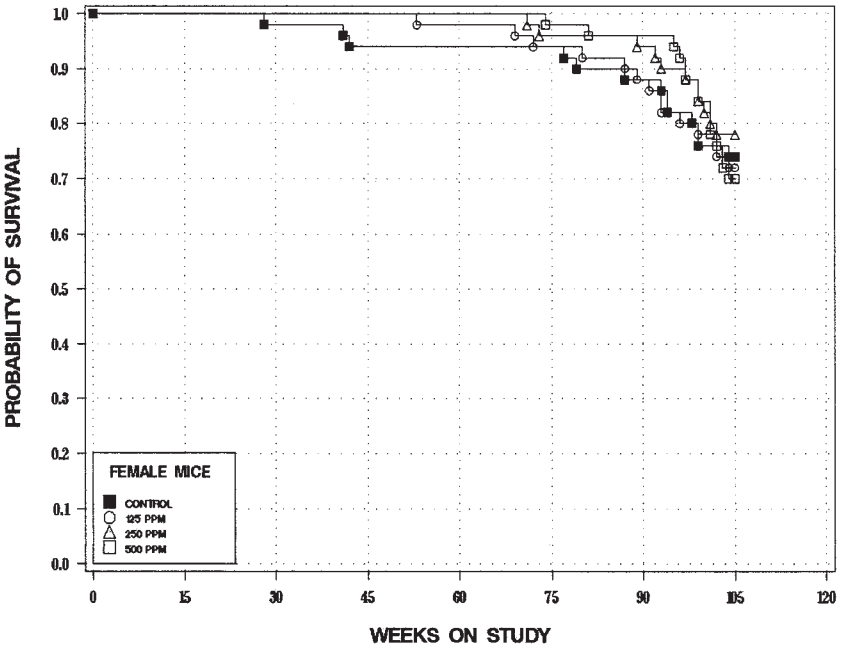




**FIGURE 4**
**Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Cumene by Inhalation for 2 Years**

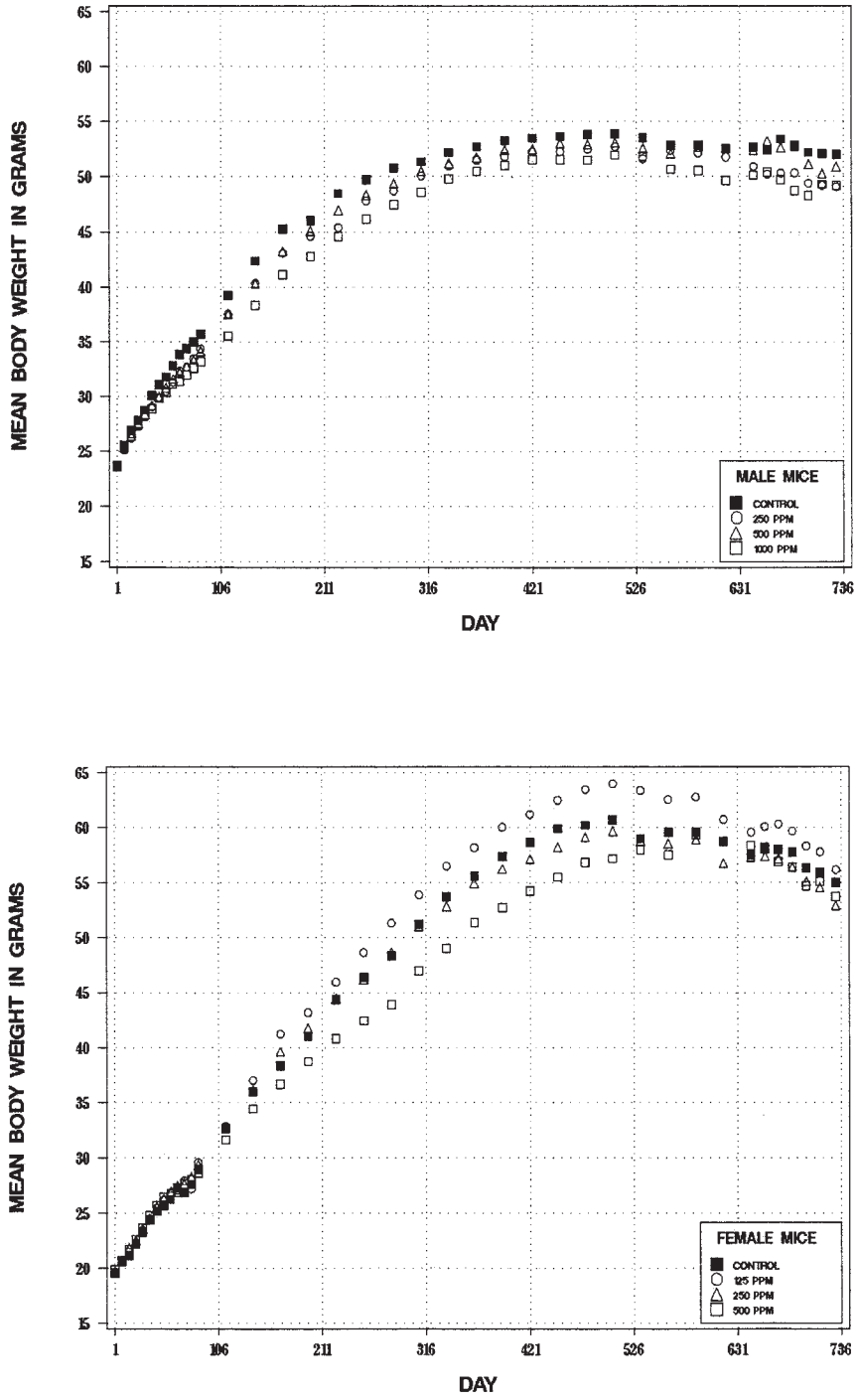



FIGURE 5
Growth Curves for Male and Female Mice
Exposed to Cumene by Inhalation for 2 Years

**TABLE 20**
**Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of Cumene**

| Days on Study | Chamber Control | | 250 ppm | | | 500 ppm | | | 1,000 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 | 23.7 | 50 | 23.8 | 100 | 50 | 23.7 | 100 | 50 | 23.6 | 99 | 50 |
| 8 | 25.6 | 50 | 25.1 | 98 | 50 | 25.5 | 100 | 50 | 25.3 | 99 | 50 |
| 15 | 26.9 | 50 | 26.2 | 97 | 50 | 26.4 | 98 | 50 | 26.6 | 99 | 50 |
| 22 | 27.8 | 50 | 27.2 | 98 | 50 | 27.5 | 99 | 50 | 27.3 | 98 | 50 |
| 29 | 28.7 | 50 | 28.1 | 98 | 50 | 28.4 | 99 | 50 | 28.3 | 98 | 50 |
| 36 | 30.1 | 50 | 29.0 | 97 | 50 | 29.2 | 97 | 50 | 28.9 | 96 | 50 |
| 43 | 31.1 | 50 | 29.9 | 96 | 50 | 30.0 | 96 | 50 | 29.8 | 96 | 50 |
| 50 | 31.8 | 50 | 30.5 | 96 | 50 | 30.8 | 97 | 50 | 30.3 | 96 | 50 |
| 57 | 32.8 | 50 | 31.3 | 95 | 50 | 31.6 | 96 | 50 | 31.1 | 95 | 50 |
| 64 | 33.8 | 50 | 32.3 | 95 | 50 | 32.2 | 95 | 50 | 31.4 | 93 | 50 |
| 71 | 34.4 | 50 | 32.7 | 95 | 50 | 32.7 | 95 | 50 | 32.0 | 93 | 50 |
| 78 | 35.0 | 50 | 33.4 | 96 | 50 | 33.4 | 95 | 50 | 32.6 | 93 | 50 |
| 85 | 35.6 | 50 | 34.3 | 96 | 50 | 34.1 | 96 | 50 | 33.2 | 93 | 50 |
| 113 | 39.2 | 50 | 37.5 | 96 | 50 | 37.5 | 96 | 50 | 35.5 | 91 | 50 |
| 141 | 42.4 | 50 | 40.3 | 95 | 50 | 40.3 | 95 | 50 | 38.3 | 90 | 50 |
| 169 | 45.3 | 50 | 43.1 | 95 | 50 | 43.2 | 96 | 50 | 41.1 | 91 | 50 |
| 197 | 46.0 | 50 | 44.6 | 97 | 50 | 45.1 | 98 | 50 | 42.8 | 93 | 50 |
| 225 | 48.5 | 50 | 45.4 | 94 | 50 | 47.0 | 97 | 50 | 44.6 | 92 | 50 |
| 253 | 49.7 | 50 | 47.8 | 96 | 50 | 48.3 | 97 | 50 | 46.2 | 93 | 50 |
| 281 | 50.8 | 50 | 48.7 | 96 | 50 | 49.4 | 97 | 50 | 47.5 | 94 | 50 |
| 309 | 51.4 | 50 | 50.1 | 98 | 50 | 50.5 | 98 | 50 | 48.6 | 95 | 50 |
| 337 | 52.2 | 50 | 51.0 | 98 | 50 | 51.2 | 98 | 50 | 49.8 | 95 | 50 |
| 365 | 52.7 | 50 | 51.5 | 98 | 50 | 51.7 | 98 | 50 | 50.5 | 96 | 50 |
| 393 | 53.2 | 50 | 51.8 | 97 | 50 | 52.4 | 98 | 49 | 51.0 | 96 | 49 |
| 421 | 53.5 | 50 | 52.2 | 98 | 50 | 52.5 | 98 | 49 | 51.5 | 96 | 47 |
| 449 | 53.6 | 50 | 52.3 | 98 | 50 | 53.0 | 99 | 49 | 51.6 | 96 | 47 |
| 477 | 53.8 | 50 | 52.5 | 98 | 49 | 53.0 | 98 | 49 | 51.5 | 96 | 46 |
| 505 | 53.9 | 50 | 52.7 | 98 | 48 | 53.1 | 99 | 48 | 52.0 | 96 | 45 |
| 533 | 53.5 | 50 | 51.6 | 96 | 48 | 52.6 | 98 | 47 | 51.8 | 97 | 44 |
| 561 | 52.8 | 49 | 52.4 | 99 | 46 | 52.1 | 99 | 47 | 50.7 | 96 | 43 |
| 589 | 52.8 | 48 | 52.1 | 99 | 46 | 52.7 | 100 | 45 | 50.6 | 96 | 42 |
| 617 | 52.5 | 48 | 51.8 | 99 | 44 | 52.5 | 100 | 44 | 49.6 | 95 | 39 |
| 645 | 52.7 | 45 | 50.9 | 97 | 43 | 52.4 | 100 | 43 | 50.1 | 95 | 36 |
| 659 | 52.4 | 45 | 50.2 | 96 | 41 | 53.2 | 102 | 41 | 50.4 | 96 | 35 |
| 673 | 53.4 | 40 | 50.3 | 94 | 40 | 52.6 | 99 | 39 | 49.7 | 93 | 34 |
| 687 | 52.8 | 40 | 50.3 | 95 | 38 | 52.7 | 100 | 37 | 48.7 | 92 | 32 |
| 701 | 52.2 | 39 | 49.4 | 95 | 36 | 51.1 | 98 | 36 | 48.3 | 93 | 27 |
| 715 | 52.1 | 39 | 49.2 | 95 | 35 | 50.3 | 97 | 34 | 49.3 | 95 | 24 |
| **Mean for weeks** | | | | | | | | | | | |
| 1-13 | 30.6 | | 29.5 | 96 | | 29.7 | 97 | | 29.3 | 96 | |
| 14-52 | 47.3 | | 45.4 | 96 | | 45.8 | 97 | | 43.8 | 93 | |
| 53-103 | 53.0 | | 51.3 | 97 | | 52.4 | 99 | | 50.5 | 95 | |

**TABLE 21**
**Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Cumene**

| Days on Study | Chamber Control | | 125 ppm | | | 250 ppm | | | 500 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 | 19.6 | 50 | 19.7 | 100 | 50 | 19.8 | 101 | 50 | 19.8 | 101 | 50 |
| 8 | 20.7 | 50 | 20.6 | 99 | 50 | 20.6 | 99 | 50 | 20.7 | 100 | 50 |
| 15 | 21.2 | 50 | 21.5 | 101 | 50 | 21.9 | 103 | 50 | 21.7 | 103 | 50 |
| 22 | 22.2 | 50 | 22.3 | 101 | 50 | 22.5 | 102 | 50 | 22.6 | 102 | 50 |
| 29 | 23.3 | 50 | 23.4 | 101 | 50 | 23.6 | 101 | 50 | 23.7 | 102 | 50 |
| 36 | 24.4 | 50 | 24.5 | 100 | 50 | 24.6 | 101 | 50 | 24.8 | 102 | 50 |
| 43 | 25.2 | 50 | 25.5 | 101 | 50 | 25.6 | 101 | 50 | 25.7 | 102 | 50 |
| 50 | 25.7 | 50 | 26.2 | 102 | 50 | 26.3 | 102 | 50 | 26.5 | 103 | 50 |
| 57 | 26.3 | 50 | 26.6 | 101 | 50 | 27.0 | 103 | 50 | 26.8 | 102 | 50 |
| 64 | 27.3 | 50 | 27.1 | 99 | 50 | 27.5 | 101 | 50 | 26.9 | 99 | 50 |
| 71 | 26.9 | 50 | 27.9 | 104 | 50 | 27.7 | 103 | 50 | 27.6 | 103 | 50 |
| 78 | 27.6 | 50 | 27.2 | 99 | 50 | 28.3 | 103 | 50 | 28.1 | 102 | 50 |
| 85 | 28.9 | 50 | 29.6 | 102 | 50 | 29.4 | 102 | 50 | 28.6 | 99 | 50 |
| 113 | 32.7 | 50 | 32.9 | 101 | 50 | 32.7 | 100 | 50 | 31.6 | 97 | 50 |
| 141 | 36.0 | 50 | 37.0 | 103 | 50 | 36.2 | 101 | 50 | 34.4 | 96 | 50 |
| 169 | 38.3 | 50 | 41.2 | 108 | 50 | 39.6 | 103 | 50 | 36.7 | 96 | 50 |
| 197 | 41.0 | 49 | 43.2 | 105 | 50 | 41.7 | 102 | 50 | 38.7 | 94 | 50 |
| 225 | 44.4 | 49 | 46.0 | 104 | 50 | 44.5 | 100 | 50 | 40.8 | 92 | 50 |
| 253 | 46.4 | 49 | 48.7 | 105 | 50 | 46.2 | 100 | 50 | 42.4 | 92 | 50 |
| 281 | 48.3 | 49 | 51.3 | 106 | 50 | 48.7 | 101 | 50 | 43.9 | 91 | 50 |
| 309 | 51.3 | 47 | 53.9 | 105 | 50 | 51.0 | 100 | 50 | 47.0 | 92 | 50 |
| 337 | 53.7 | 47 | 56.5 | 105 | 50 | 52.9 | 98 | 50 | 49.0 | 91 | 50 |
| 365 | 55.6 | 47 | 58.2 | 105 | 50 | 55.0 | 99 | 50 | 51.4 | 92 | 50 |
| 393 | 57.3 | 47 | 60.0 | 105 | 49 | 56.2 | 98 | 50 | 52.7 | 92 | 50 |
| 421 | 58.6 | 47 | 61.2 | 104 | 49 | 57.1 | 97 | 50 | 54.3 | 93 | 50 |
| 449 | 59.9 | 47 | 62.5 | 104 | 49 | 58.2 | 97 | 50 | 55.5 | 93 | 50 |
| 477 | 60.2 | 47 | 63.5 | 105 | 49 | 59.1 | 98 | 50 | 56.8 | 94 | 50 |
| 505 | 60.7 | 47 | 64.0 | 106 | 47 | 59.7 | 98 | 49 | 57.2 | 94 | 50 |
| 533 | 59.0 | 47 | 63.4 | 107 | 47 | 58.7 | 100 | 48 | 58.0 | 98 | 49 |
| 561 | 59.6 | 45 | 62.5 | 105 | 46 | 58.5 | 98 | 48 | 57.5 | 97 | 49 |
| 589 | 59.6 | 45 | 62.7 | 105 | 46 | 58.9 | 99 | 48 | 59.3 | 100 | 48 |
| 617 | 58.7 | 44 | 60.7 | 103 | 45 | 56.7 | 97 | 48 | 58.7 | 100 | 48 |
| 645 | 57.5 | 44 | 59.6 | 104 | 43 | 57.3 | 100 | 46 | 58.4 | 102 | 48 |
| 659 | 58.1 | 41 | 60.1 | 104 | 41 | 57.4 | 99 | 45 | 58.2 | 100 | 48 |
| 673 | 58.0 | 41 | 60.3 | 104 | 40 | 57.2 | 99 | 45 | 56.9 | 98 | 46 |
| 687 | 57.8 | 40 | 59.7 | 103 | 40 | 56.4 | 98 | 43 | 56.4 | 98 | 44 |
| 701 | 56.3 | 38 | 58.3 | 104 | 39 | 55.1 | 98 | 41 | 54.7 | 97 | 42 |
| 715 | 56.0 | 38 | 57.8 | 103 | 37 | 54.6 | 98 | 39 | 55.1 | 98 | 38 |
| **Mean for weeks** | | | | | | | | | | | |
| 1-13 | 24.6 | | 24.8 | 101 | | 25.0 | 102 | | 24.9 | 101 | |
| 14-52 | 43.6 | | 45.6 | 105 | | 43.7 | 100 | | 40.5 | 93 | |
| 53-103 | 58.3 | | 60.9 | 104 | | 57.3 | 98 | | 56.3 | 97 | |

### *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 lung, nose, liver, forestomach, spleen, and thyroid 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 C for male mice and Appendix D for female mice.

*Lung:* The incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma or carcinoma (combined) in all exposed groups of mice occurred with positive trends, were significantly greater than those in the chamber controls, and exceeded the ranges for historical chamber controls in inhalation studies and historical controls (all routes) (Tables 22, C2, C3a, D2, and D3a). Significantly increased incidences of multiple alveolar/bronchiolar adenoma and multiple alveolar/bronchiolar carcinoma occurred in all exposed groups. Microscopically, most of the alveolar/bronchiolar adenomas had papillary patterns (Plates 8 and 9). Tumor margins were usually well-demarcated with compression of the surrounding parenchyma. Alveolar/bronchiolar carcinomas varied from well-differentiated neoplasms with papillary patterns to poorly circumscribed, infiltrative tumors consisting of densely packed pleomorphic cells having multiple layers of nuclei (Plates 10 and 11). Often, prominent alveolar infiltrates of macrophages and occasional multinucleate giant cells were associated with these carcinomas.

The incidences of alveolar epithelial bronchiole metaplasia and bronchiole hyperplasia were significantly increased in all exposed groups of mice, and the severity increased in all exposed groups (Tables 22, C4, and D4). Microscopically, alveolar epithelial bronchiole metaplasia occurs when the flat epithelium of the alveolar ducts and adjacent alveolar septa is replaced by cells similar in appearance to those normally lining terminal bronchioles. Minimal to marked alveolar epithelial bronchiole metaplasia in the exposed mice was characterized by increases in the numbers of plump cuboidal epithelial cells having large hyperchromatic nuclei that lined the alveolar walls adjacent to and extending from the terminal bronchioles (Plates 12 and 13). Minimal to marked bronchiole hyperplasia resulted in papillary projections into the bronchiolar lumens by cuboidal epithelial cells lining the bronchioles (Plate 14). When these lesions had marked hyperplasia, the epithelium filled the bronchi and resembled early alveolar/bronchiolar adenomas.

*Nose:* Exposure to cumene resulted in increased incidences of olfactory epithelium atrophy, olfactory epithelium basal cell hyperplasia, olfactory epithelium atypical hyperplasia, olfactory epithelium glands hyperplasia, respiratory epithelium squamous metaplasia, and suppurative inflammation (Tables 23, C4, and D4). Although nasal lesions were seen in all three nasal cavity levels that are routinely examined in NTP toxicity and carcinogenicity studies, they predominantly occurred in Level III and to a lesser extent in Level I.

The incidences of olfactory epithelium atrophy in all exposed groups of males and 125 and 500 ppm females were significantly greater than those in the chamber controls (Tables 23, C4, and D4). Microscopically, minimal to mild olfactory epithelium atrophy consisted of small focal lesions involving primarily the epithelium lining the dorsal meatus in Level III nasal sections and occasionally the ethmoid turbinates. The epithelium was thin, with decreased numbers of olfactory neurons (Plate 15); often, metaplasia of the olfactory epithelium to ciliated columnar epithelium and diffuse loss of Bowman's glands in the adjacent lamina propria occurred.

The incidences of basal cell hyperplasia of the olfactory epithelium were significantly increased in 500 and 1,000 ppm males and 250 and 500 ppm females (Tables 23, C4, and D4). Minimal to moderate basal cell hyperplasia of the olfactory epithelium occurred in Level III nasal sections. Minimal hyperplasia was characterized by increased numbers of basal cells crowded along the basement membrane of the olfactory epithelium. In addition to increased basal cells along the basement membrane, mild lesions had infiltration of the adjacent lamina propria by basal cells, with occasional formation of small rosettes within the epithelium. In moderate lesions, the increase in basal cells was accompanied by disruption of the olfactory epithelium.

The incidences of atypical hyperplasia of the olfactory epithelium were significantly increased in groups of mice exposed to 500 ppm or greater (Tables 23, C4, and D4). Minimal to moderate atypical hyperplasia of the olfactory epithelium consisted of proliferation of atypical basal cells with disruption and infiltration of the adjacent lamina propria of ethmoid turbinates

**TABLE 22**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Male** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Alveolar Epithelium, Bronchiole, Metaplasia[a] | 5 (1.4)[b] | 43** (2.9) | 42** (3.1) | 39** (3.0) |
| Bronchiole, Hyperplasia | 0 | 11** (2.1) | 17** (3.2) | 18** (2.8) |
| Alveolar/bronchiolar Adenoma, Multiple | 1 | 12** | 15** | 20** |
| Alveolar/bronchiolar Adenoma (includes multiple)[c] | | | | |
| Overall rate[d] | 13/50 (26%) | 31/50 (62%) | 31/50 (62%) | 29/50 (58%) |
| Adjusted rate[e] | 27.5% | 66.7% | 66.9% | 67.9% |
| Terminal rate[f] | 10/38 (26%) | 25/34 (74%) | 23/30 (77%) | 20/23 (87%) |
| First incidence (days) | 628 | 551 | 512 | 480 |
| Poly-3 test[g] | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Alveolar/bronchiolar Carcinoma, Multiple | 0 | 8** | 20** | 17** |
| Alveolar/bronchiolar Carcinoma (includes multiple)[h] | | | | |
| Overall rate | 9/50 (18%) | 19/50 (38%) | 32/50 (64%) | 33/50 (66%) |
| Adjusted rate | 19.1% | 41.5% | 70.5% | 71.3% |
| Terminal rate | 6/38 (16%) | 15/34 (44%) | 25/30 (83%) | 12/23 (52%) |
| First incidence (days) | 631 | 551 | 565 | 420 |
| Poly-3 test | P<0.001 | P=0.014 | P<0.001 | P<0.001 |
| Alveolar/bronchiolar Adenoma or Carcinoma[i] | | | | |
| Overall rate | 19/50 (38%) | 38/50 (76%) | 42/50 (84%) | 43/50 (86%) |
| Adjusted rate | 39.8% | 81.4% | 89.5% | 92.1% |
| Terminal rate | 14/38 (37%) | 31/34 (91%) | 30/30 (100%) | 21/23 (91%) |
| First incidence (days) | 628 | 551 | 512 | 420 |
| Poly-3 test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Female** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Alveolar Epithelium, Bronchiole, Metaplasia | 0 | 42** (2.6) | 49** (2.9) | 47** (3.3) |
| Bronchiole, Hyperplasia | 0 | 17** (2.7) | 10** (2.8) | 14** (2.8) |
| Alveolar/bronchiolar Adenoma, Multiple | 0 | 13** | 20** | 30** |
| Alveolar/bronchiolar Adenoma (includes multiple)[j] | | | | |
| Overall rate | 1/50 (2%) | 26/50 (52%) | 36/50 (72%) | 38/50 (76%) |
| Adjusted rate | 2.3% | 56.3% | 74.5% | 77.9% |
| Terminal rate | 1/37 (3%) | 21/36 (58%) | 31/39 (80%) | 29/35 (83%) |
| First incidence (days) | 731 (T) | 555 | 495 | 565 |
| Poly-3 test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |

**TABLE 22**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Female** (continued) | | | | |
| Alveolar/bronchiolar Carcinoma, Multiple | 0 | 6* | 7** | 19** |
| Alveolar/bronchiolar Carcinoma (includes multiple)[k] | | | | |
| Overall rate | 3/50 (6%) | 16/50 (32%) | 20/50 (40%) | 34/50 (68%) |
| Adjusted rate | 6.7% | 35.3% | 41.9% | 69.5% |
| Terminal rate | 2/37 (5%) | 13/36 (36%) | 15/39 (39%) | 24/35 (69%) |
| First incidence (days) | 533 | 646 | 618 | 513 |
| Poly-3 test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Alveolar/bronchiolar Adenoma or Carcinoma[l] | | | | |
| Overall rate | 4/50 (8%) | 31/50 (62%) | 42/50 (84%) | 46/50 (92%) |
| Adjusted rate | 8.9% | 66.8% | 86.0% | 92.4% |
| Terminal rate | 3/37 (8%) | 25/36 (69%) | 34/39 (87%) | 33/35 (94%) |
| First incidence (days) | 533 | 555 | 495 | 513 |
| Poly-3 test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |

(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 control groups (mean ± standard deviation): 92/449 (20.5% ± 5.3%), range 12%-26%; all routes: 263/1,498 (17.9% ± 6.1%), range 6%-28%
[d] Number of animals with neoplasm per number of animals with lung examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] 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 comparison between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence for inhalation studies: 64/449 (14.2% ± 4.6%), range 10%-24%; all routes: 161/1,498 (10.9% ± 5.6%), range 2%-24%
[i] Historical incidence for inhalation studies: 146/449 (32.5% ± 5.9%), range 26%-44%; all routes: 401/1,498 (27.2% ± 7.8%), range 12%-44%
[j] Historical incidence for inhalation studies: 19/449 (4.2% ± 2.5%), range 2%-8%; all routes: 77/1,596 (4.9% ± 2.7%), range 0%-12%
[k] Historical incidence for inhalation studies: 15/449 (3.4% ± 3.9%), range 0%-12%; all routes: 57/1,596 (3.6% ± 3.1%), range 0%-12%
[l] Historical incidence for inhalation studies: 34/449 (7.6% ± 4.0%), range 2%-14%; all routes: 129/1,596 (8.2% ± 3.9%), range 2%-18%

by irregular polyhedral cells. These cells had hyperchromatic, round to oval nuclei and scant basophilic cytoplasm. Frequently, rosettes of columnar cells with basally located nuclei around a central lumen were formed. Moderate atypical basal cell hyperplasia in four 1,000 ppm male mice had features of preneoplastic lesions (Plate 16).

The incidences of hyperplasia of olfactory epithelium glands were significantly increased in all exposed groups of males and in 500 ppm females (Tables 23, C4, and D4). Minimal focal hyperplasia of olfactory epithelium glands consisted of a few dilated Bowman's glands lined by multiple layers of epithelial cells.

The incidence of squamous metaplasia of the respiratory epithelium was significantly increased in 500 ppm females (Tables 23 and D4). Minimal to mild squamous metaplasia of the respiratory epithelium was characterized by replacement of the normal cuboidal to columnar epithelium along the tips of the turbinates in nasal section Level I by squamous epithelial cells.

**TABLE 23**
**Incidences of Selected Nonneoplastic Lesions of the Nose in Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Male** | | | | |
| Number Examined Microscopically | 50 | 50 | 49 | 48 |
| Olfactory Epithelium, Atrophy[a] | 4 (1.3)[b] | 13* (1.1) | 11* (1.2) | 38** (1.4) |
| Olfactory Epithelium, Hyperplasia, Basal Cell | 0 | 0 | 15** (1.0) | 33** (1.1) |
| Olfactory Epithelium, Hyperplasia, Atypical | 0 | 0 | 5* (1.6) | 11** (1.7) |
| Olfactory Epithelium, Glands, Hyperplasia | 3 (1.0) | 11* (1.0) | 9* (1.1) | 23** (1.0) |
| Inflammation, Suppurative | 2 (2.0) | 2 (1.5) | 9* (1.1) | 6 (1.5) |
| | **Chamber Control** | **125 ppm** | **250 ppm** | **500 ppm** |
| **Female** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Olfactory Epithelium, Atrophy | 4 (1.0) | 11* (1.1) | 9 (1.1) | 18** (1.2) |
| Olfactory Epithelium, Hyperplasia, Basal Cell | 0 | 1 (1.0) | 11** (1.0) | 25** (1.1) |
| Olfactory Epithelium, Hyperplasia, Atypical | 0 | 0 | 2 (1.0) | 10** (1.2) |
| Olfactory Epithelium, Glands, Hyperplasia | 1 (1.0) | 4 (1.0) | 4 (1.0) | 11** (1.0) |
| Respiratory Epithelium, Metaplasia, Squamous | 0 | 0 | 1 (2.0) | 6* (1.2) |
| Inflammation, Suppurative | 0 | 1 (1.0) | 3 (1.3) | 7* (1.3) |

* 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

The incidences of suppurative inflammation were significantly increased in 500 ppm males and females (Tables 23, C4, and D4). Minimal to mild suppurative inflammation resulted from infiltration of the lamina propria by various inflammatory cells, predominantly neutrophils, and was associated with necrosis or squamous metaplasia of the overlying epithelium.

*Liver:* In females, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) occurred with positive trends and were significantly increased in the 500 ppm group; incidences of these lesions in all exposed groups of females and the incidence of hepatocellular carcinoma in 500 ppm females exceeded the historical ranges for chamber controls but were generally within the historical ranges for controls (all routes) (Tables 24, D2, and D3b). Microscopically, the hepatocellular adenomas and hepatocellular carcinomas had the typical appearance of these tumors as reported in B6C3F1 mice. Hepatocellular adenomas were usually discrete masses having solid growth patterns that caused compression of the surrounding normal hepatic parenchyma. They consisted of hepatocytes having clear, eosinophilic, or basophilic cytoplasm, and were sometimes difficult to distinguish from hepatocellular foci. However, the lack of normal lobular architecture and the presence of plates of neoplastic hepatocytes that intersected the surrounding normal liver plates at sharp angles, rather than merging with them as seen in foci, were characteristics used to differentiate adenomas from foci. Hepatocellular carcinomas were large, poorly demarcated masses that generally had irregular borders due to growth into the surrounding normal parenchyma. The neoplastic hepatocytes often were somewhat atypical in appearance, but the major distinguishing feature of carcinomas was the presence of abnormal patterns of growth. The most common abnormal growth pattern was formation of trabeculae of neoplastic hepatocytes that were three or more cell layers thick, while less commonly, the neoplastic cells formed glandular structures or solid masses. Several growth patterns were often seen within a single

**TABLE 24**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Male** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Eosinophilic Focus[a] | 6 | 5 | 16** | 14* |
| Hepatocellular Adenoma, Multiple | 17 | 20 | 22 | 26 |
| Hepatocellular Adenoma (includes multiple)[b] | 34 | 33 | 37 | 35 |
| Hepatocellular Carcinoma, Multiple | 3 | 1 | 4 | 7 |
| Hepatocellular Carcinoma (includes multiple)[c] | 13 | 18 | 21 | 17 |
| Hepatocellular Adenoma or Carcinoma[d] | | | | |
| Overall rate[e] | 40/50 (80%) | 42/50 (84%) | 43/50 (86%) | 41/50 (82%) |
| Adjusted rate[f] | 81.0% | 85.8% | 87.2% | 87.1% |
| Terminal rate[g] | 30/38 (79%) | 28/34 (82%) | 26/30 (87%) | 20/23 (87%) |
| First incidence (days) | 551 | 453 | 381 | 391 |
| Poly-3 test[h] | P=0.250 | P=0.355 | P=0.284 | P=0.286 |

| | Chamber Control | 125 ppm | 250ppm | 500 ppm |
|---|---|---|---|---|
| **Female** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Eosinophilic Focus | 8 | 11 | 7 | 14 |
| Hepatocellular Adenoma, Multiple | 9 | 13 | 9 | 10 |
| Hepatocellular Adenoma (includes multiple)[i] | | | | |
| Overall rate | 18/50 (36%) | 23/50 (46%) | 27/50 (54%)[j] | 29/50 (58%) |
| Adjusted rate | 40.5% | 50.0% | 56.4% | 59.8% |
| Terminal rate | 17/37 (46%) | 17/36 (47%) | 22/39 (56%) | 19/35 (54%) |
| First incidence (days) | 654 | 609 | 618 | 662 |
| Poly-3 test | P=0.040 | P=0.243 | P=0.091 | P=0.046 |
| Hepatocellular Carcinoma, Multiple | 2 | 1 | 2 | 0 |
| Hepatocellular Carcinoma (includes multiple)[k] | 10 | 7 | 6 | 12 |

**TABLE 24**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Female** (continued) | | | | |
| Hepatocellular Adenoma or Carcinoma[l] | | | | |
| Overall rate | 25/50 (50%) | 26/50 (52%) | 29/50 (58%)[j] | 36/50 (72%) |
| Adjusted rate | 55.6% | 56.5% | 60.4% | 74.1% |
| Terminal rate | 22/37 (60%) | 20/36 (56%) | 23/39 (59%) | 25/35 (71%) |
| First incidence (days) | 607 | 609 | 618 | 662 |
| Poly-3 test | P=0.024 | P=0.549 | P=0.395 | P=0.043 |

* 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] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 196/449 (43.7% ± 10.9%), range 30%-68%; all routes: 633/1,496 (43.3% ± 14.2%), range 14%-70%
[c] Historical incidence for inhalation studies: 107/449 (23.8% ± 4.6%), range 18%-32%; all routes: 382/1,496 (26.0% ± 9.1%), range 8%-48%
[d] Historical incidence for inhalation studies: 264/449 (58.8% ± 9.6%), range 50%-80%; all routes: 874/1,496 (59.6% ± 15.4%), range 20%-85%
[e] Number of animals with neoplasm per number of animals with liver examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[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 comparison 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 inhalation studies: 109/447 (24.4% ± 8.7%), range 12%-36%; all routes: 402/1,593 (25.8% ± 15.8%), range 2%-62%
[j] One animal with adenoma also had hepatoblastoma.
[k] Historical incidence for inhalation studies: 48/447 (10.7% ± 4.1%), range 6%-20%; all routes: 159/1,593 (10.2% ± 6.6%), range 0%-28%
[l] Historical incidence for inhalation studies: 145/447 (32.4% ± 8.8%), range 22%-50%; all routes: 505/1,593 (32.4% ± 17.5%), range 8%-64%

neoplasm. Areas of hemorrhage or necrosis were sometimes present. Metastases of carcinomas to the lungs occurred in all groups of mice of both sexes and were often multiple.

The incidences of eosinophilic foci were significantly increased in male mice exposed to 500 or 1,000 ppm (Tables 24 and C4). Eosinophilic foci consisted of well-demarcated collections of enlarged hepatocytes with abundant, dark, homogeneous, eosinophilic cytoplasm. These hepatocytes were arranged in normal hepatic cords that merged with the surrounding normal hepatocytes. Usually, little or no compression of the surrounding normal hepatocytes occurred, although some degree of compression was occasionally evident in larger foci. Eosinophilic foci, hepatocellular adenomas, and hepatocellular carcinomas are thought to represent a morphologic continuum.

*Forestomach:* The incidences of epithelial hyperplasia were significantly increased in groups of males exposed to 500 or 1,000 ppm, and the incidences of ulceration and inflammation were significantly increased in the 1,000 ppm group (Tables 25 and C4). These three lesions were often present in the forestomach of the same mouse. Epithelial hyperplasia was characterized by a diffuse thickening of the squamous epithelium due to increased numbers of cell layers, primarily of prickle cells. Ulcers of the forestomach resulted after damage to the mucosal surface, with loss of squamous epithelium extending through the basement membrane. The ulcers were usually accompanied by inflammation. The

**TABLE 25**
**Incidences of Nonneoplastic Lesions of the Forestomach in Male Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| Number Examined Microscopically | 50 | 50 | 50 | 49 |
| Epithelium, Hyperplasia[a] | 2 (2.0)[b] | 7 (2.1) | 8* (2.3) | 13** (2.1) |
| Ulcer | 1 (3.0) | 4 (2.8) | 6 (2.8) | 6* (2.8) |
| Inflammation | 0 | 2 (2.0) | 1 (2.0) | 5* (2.0) |

* 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

margins of the ulcers often had epithelial hyperplasia. Inflammation of the forestomach had variable numbers of mixed inflammatory cells, with congestion and various degrees of increased fibrous connective tissue.

*Spleen:* In males, hemangiosarcoma of the spleen occurred with a significant positive trend, and the incidence in the 1,000 ppm group was significantly greater than that in the chamber control group and exceeded the historical ranges for chamber controls in inhalation studies and historical controls (all routes) (Tables 26, C2, and C3c). The incidences of hemangiosarcoma in all organs (heart, liver, urinary bladder, spleen) in male mice also increased with a statistically significant positive trend, and the incidence was significantly increased in 1,000 ppm males. The incidence of hemangiosarcoma in all organs (spleen, liver, mesentery, bone, nose, and skeletal muscle) of 500 ppm females exceeded the historical range for chamber controls in inhalation studies (Tables 26, D2, and D3c). The role of cumene exposure in increased incidences of hemangiosarcoma in male and female mice is uncertain.

Splenic hemangiosarcoma consisted of red and white pulp being replaced with vascular spaces of various sizes lined by plump, pleomorphic endothelial cells and supported by varying amounts of fibrous connective tissue.

*Thyroid gland:* Incidences of follicular cell adenoma increased with a statistically significant positive trend in males; the incidence in 1,000 ppm males was at the upper end of the historical ranges for chamber controls in inhalation studies and for historical controls (all routes) (Tables 27, C2, and C3d). The incidence of follicular cell hyperplasia was increased in 1,000 ppm males, but the increase was not significant compared to the chamber control group (Tables 27 and C4). These marginal increases were considered possibly related to cumene exposure.

The follicular cell adenomas were well-circumscribed nodules consisting of irregularly shaped follicles containing various amounts of colloid. They were formed by proliferation of hyperchromatic follicular cells and compressed, to various degrees, the adjacent follicles.

**TABLE 26**
**Incidences of Hemangiosarcoma in Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Male** | | | | |
| Hemangiosarcoma, Spleen[a] | | | | |
| Overall rate[b] | 0/50 (0%) | 0/50 (0%) | 0/49 (0%) | 4/50 (8%) |
| Adjusted rate[c] | 0.0% | 0.0% | 0.0% | 9.9% |
| Terminal rate[d] | 0/38 (0%) | 0/34 (0%) | 0/30 (0%) | 3/23 (13%) |
| First incidence (days) | —[e] | — | — | 556 |
| Poly-3 test[f] | P=0.002 | —[g] | — | P=0.045 |
| Hemangiosarcoma, All Organs[h] | | | | |
| Overall rate[i] | 0/50 (0%) | 1/50 (2%) | 2/50 (4%) | 4/50 (8%) |
| Adjusted rate | 0.0% | 2.2% | 4.5% | 9.9% |
| Terminal rate | 0/38 (0%) | 0/34 (0%) | 2/30 (7%) | 3/23 (13%) |
| First incidence (days) | — | 654 | 729 (T) | 556 |
| Poly-3 test | P=0.015 | P=0.493 | P=0.226 | P=0.045 |

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Female** | | | | |
| Number Examined Microscopically | 49 | 50 | 50 | 50 |
| Hemangiosarcoma, Spleen[j,k] | 0 | 0 | 3 | 1 |
| Hemangiosarcoma, All Organs[l] | | | | |
| Overall rate[i] | 1/50 (2%) | 3/50 (6%) | 6/50 (12%) | 1/50 (2%) |
| Adjusted rate | 2.3% | 6.6% | 12.8% | 2.1% |
| Terminal rate | 1/37 (3%) | 1/36 (3%) | 5/39 (13%) | 1/35 (3%) |
| First incidence (days) | 731 (T) | 555 | 673 | 731 (T) |
| Poly-3 test | P=0.518N | P=0.318 | P=0.066 | P=0.746N |

(T) Terminal sacrifice
[a] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 6/444 (1.4% ± 1.5%), range 0%-4%; all routes: 24/1,483 (1.7% ± 1.2%), range 0%-4%
[b] Number of animals with neoplasm per number of animals with tissue examined microscopically
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Not applicable; no neoplasms in animal group
[f] 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 comparison 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 negative trend or a lower incidence in an exposed group is indicated by **N**.
[g] Value of statistic cannot be computed.
[h] Historical incidence for inhalation studies: 21/450 (4.7% ± 3.7%), range 0%-12%; all routes: 76/1,499 (5.2% ± 3.2%), range 0%-12%
[i] Number of animals with neoplasm per number of animals necropsied
[j] Number of animals with lesion
[k] Historical incidence for inhalation studies: 6/445 (1.3% ± 1.4), range 0%-4%; all routes: 27/1,573 (1.8% ± 1.8%), range 0%-8%
[l] Historical incidence for inhalation studies: 16/449 (3.6 ± 2.2%), range 2%-8%; all routes: 71/1,598 (4.6% ± 3.2%), range 2%-16%

**TABLE 27**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Male Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| Number Examined Microscopically | 50 | 50 | 49 | 50 |
| Follicular Cell, Hyperplasia[a] | 7 (1.9)[b] | 7 (2.4) | 7 (1.7) | 11 (1.9) |
| Follicular Cell, Adenoma[c] | | | | |
| Overall rate[d] | 0/50 (0%) | 0/50 (0%) | 0/49 (0%) | 3/50 (6%) |
| Adjusted rate[e] | 0.0% | 0.0% | 0.0% | 7.5% |
| Terminal rate[f] | 0/38 (0%) | 0/34 (0%) | 0/30 (0%) | 2/23 (9%) |
| First incidence (days) | —[g] | — | — | 680 |
| Poly-3 test[h] | P=0.010 | —[i] | — | P=0.095 |

[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 control groups (mean ± standard deviation): 5/441 (1.1% ± 2.0%), range 0%-6%; all routes: 21/1,483 (1.4% ± 1.8%), range 0%-6%
[d] Number of animals with neoplasm per number of animals with thyroid gland 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 comparison 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.

## GENETIC TOXICOLOGY

Cumene (1 to 333 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535 when tested with and without induced rat or hamster liver S9 activation enzymes (Table E1). *In vivo*, cumene induced small, but significant, increases in micronucleated polychromatic erythrocytes in bone marrow of male rats treated by intraperitoneal injection (Table E2). Two trials were performed in rats. In the first trial, doses ranging from 78.13 to 2,500 mg/kg were administered three times at 24-hour intervals, and results were positive, based both on the trend ($P<0.001$) and the response at the 1,250 mg/kg dose. The data from the 2,500 mg/kg dose were excluded from analysis because only two animals survived and a minimum of three animals is required for a valid dose point. The second confirmatory trial also produced a positive response, although the trend test was not significant ($P=0.085$). Micronucleated erythrocytes were elevated at all four doses in trial 2; the responses at the 312 and 1,250 mg/kg levels were statistically significant ($P<0.006$). Because considerable toxicity was again observed at the high dose of 2,500 mg/kg, the trend was recalculated over the same dose range as for the first trial (0 to 1,250 mg/kg), and a significant P value of 0.019 was produced. The percentage of polychromatic erythrocytes in the bone marrow fluctuated unrelated to dose and likely represented variation within the normal range of 40% to 60% polychromatic erythrocytes among the total erythrocyte population in the bone marrow. In contrast to the results in male rats, no increase in micronucleated erythrocytes was observed in peripheral blood of male or female mice exposed to cumene by inhalation (62.5 to 1,000 ppm) for 3 months (Table E3). For both male and female mice, no significant changes in the percentage of polychromatic erythrocytes were observed over the exposure range tested, indicating an absence of treatment-related toxicity to the bone marrow.

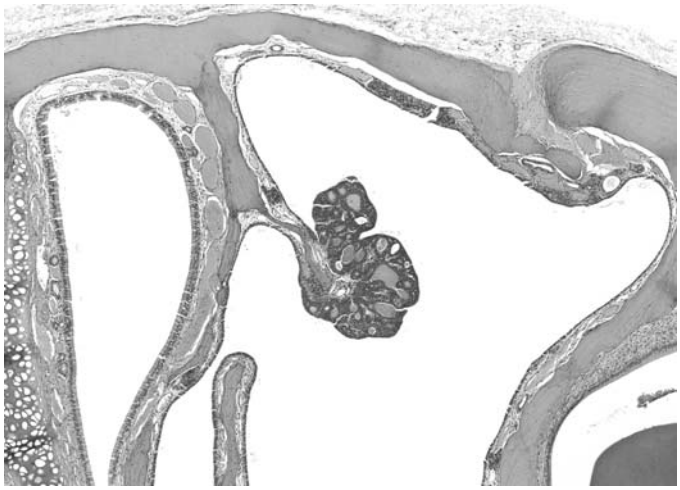


**PLATE 1**
Nasal cavity of a female F344/N rat exposed to 250 ppm cumene in the 2-year inhalation study. Adenoma arising from the respiratory epithelium lining the nasoturbinate in nasal Level I. H&E

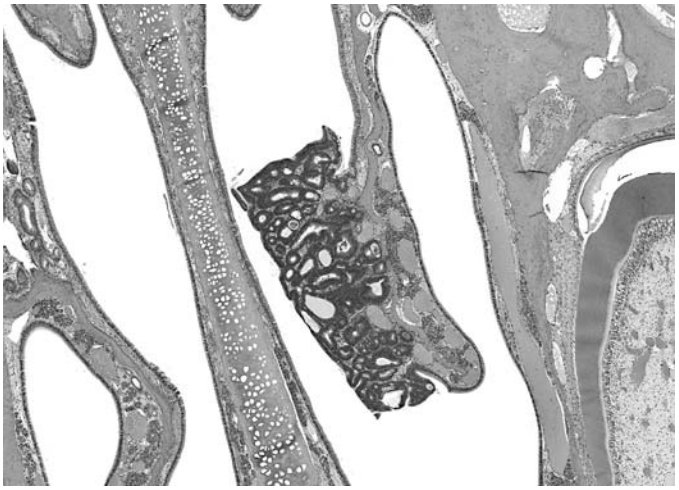


**PLATE 2**
Nasal cavity of a female F344/N rat exposed to 250 ppm cumene in the 2-year inhalation study. Adenoma arising from the respiratory epithelium lining the nasoturbinate in nasal Level II. Note the formation of acinar patterns by basophilic neoplastic cells. H&E

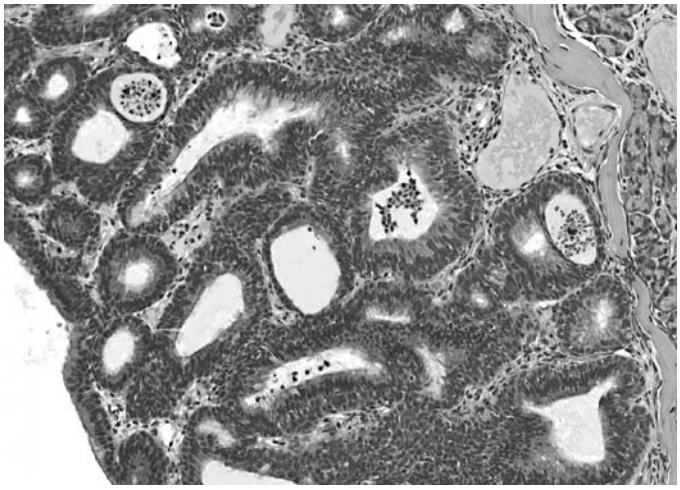


**PLATE 3**
Higher magnification of Plate 2. Note the acinar patterns formed by cords of neoplastic basophilic respiratory epithelium. H&E

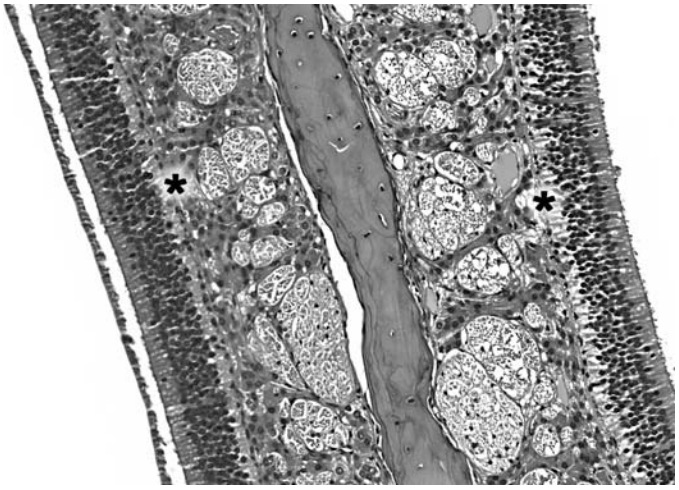


**PLATE 4**
Olfactory epithelium with a normal, orderly, thin basal cell layer (asterisks) beneath the multiple layers of neuron nuclei in nasal Level III of a male chamber control F344/N rat at 2 years in the inhalation study of cumene. H&E

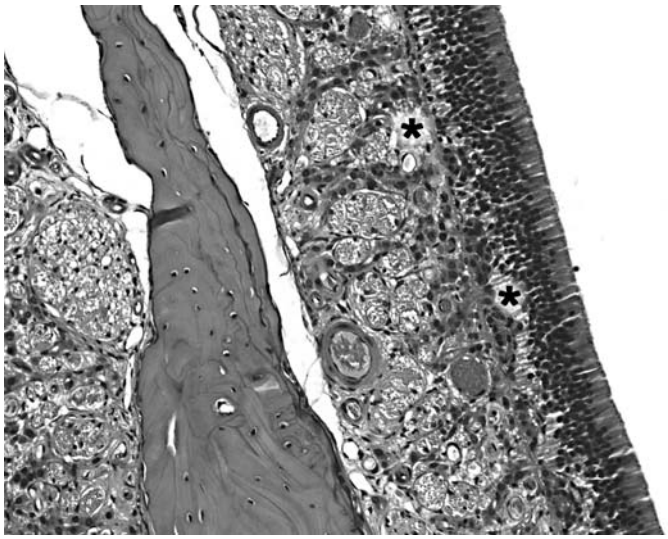


**PLATE 5**
Olfactory epithelium with minimal basal cell hyperplasia in nasal Level III of a female F344/N rat exposed to 1,000 ppm cumene in the 2-year inhalation study. Note the increased numbers of basophilic cells along the basement membrane (asterisks) under more orderly, normal appearing olfactory neuron nuclei. H&E

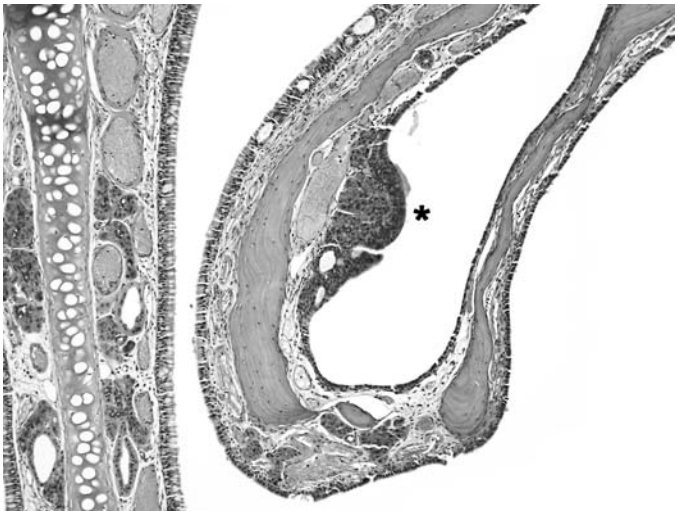


**PLATE 6**
Nasal cavity of a male F344/N rat exposed to 1,000 ppm cumene in the 2-year inhalation study. Note the localized hyperplasia of the respiratory epithelium lining the nasoturbinate in nasal Level I (asterisk). H&E

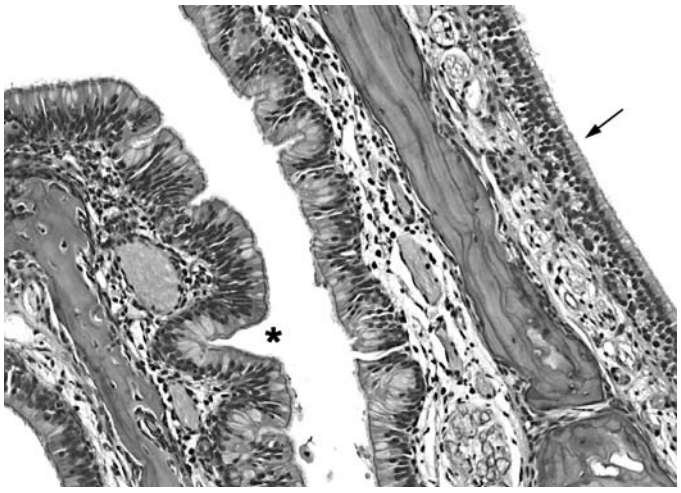


**PLATE 7**
Olfactory epithelium in nasal Level III of a male F344/N rat exposed to 500 ppm cumene in the 2-year inhalation study. Note the undulating mucosa resulting from goblet cell hyperplasia of metaplastic, respiratory-like epithelial cells (asterisk). Compare these changes with the more normal olfactory epithelium lining the opposite surface of the nasal septum (arrow). H&E

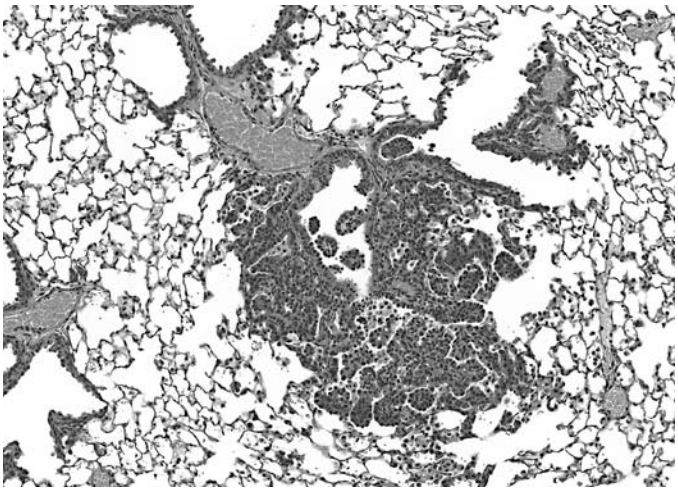


**PLATE 8**
Lung of a female B6C3F1 mouse exposed to 250 ppm cumene in the 2-year inhalation study. An alveolar/bronchiolar adenoma arising at the alveolar-bronchiolar junction with extension into adjacent alveoli. Note the papillary patterns. H&E

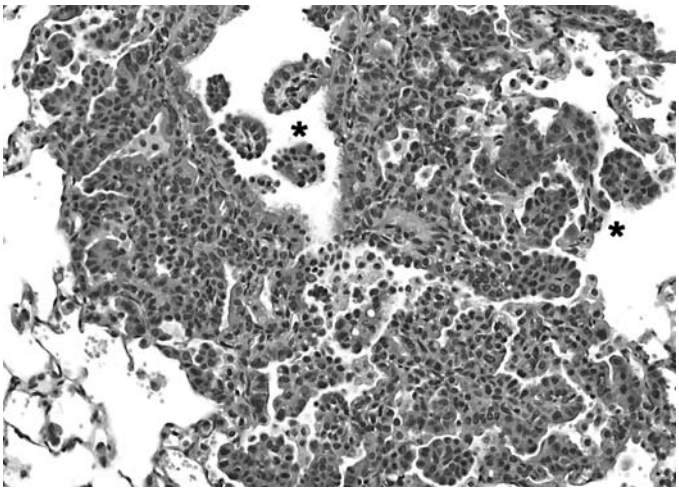


**PLATE 9**
Higher magnification of Plate 8. Note the papillary patterns formed by cuboidal neoplastic epithelial cells (asterisks). H&E

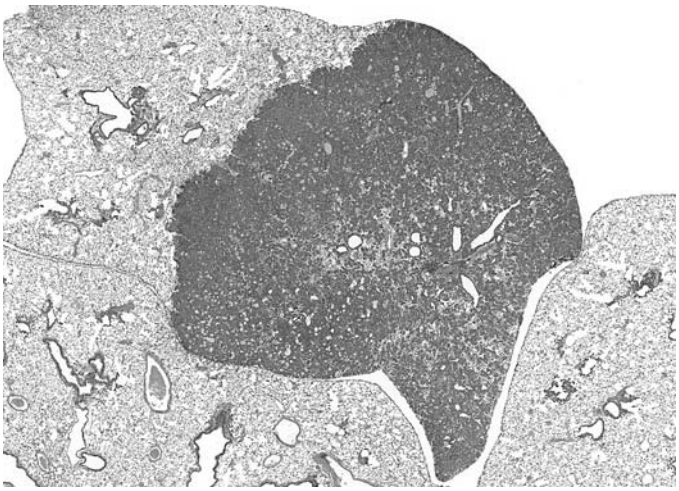


**PLATE 10**
Lung of a male B6C3F1 mouse exposed to 500 ppm cumene in the 2-year inhalation study. This alveolar/bronchiolar carcinoma involves a large portion of the affected lobe. H&E

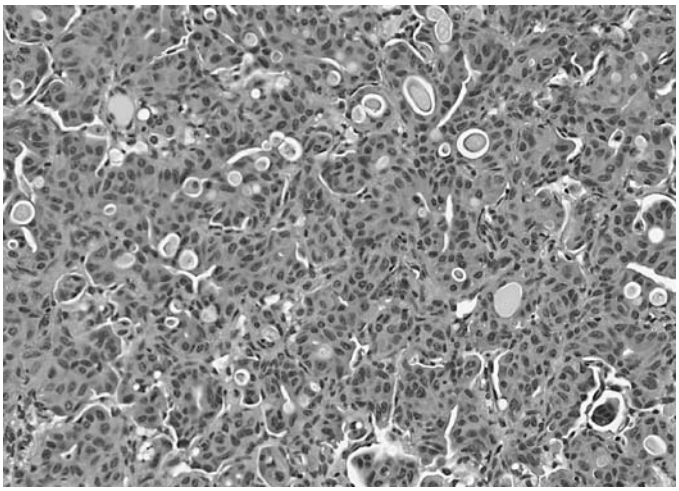


**PLATE 11**
Higher magnification of Plate 10. Note that although the neoplastic cells are anaplastic and pleomorphic, cuboidal epithelial features persist. H&E

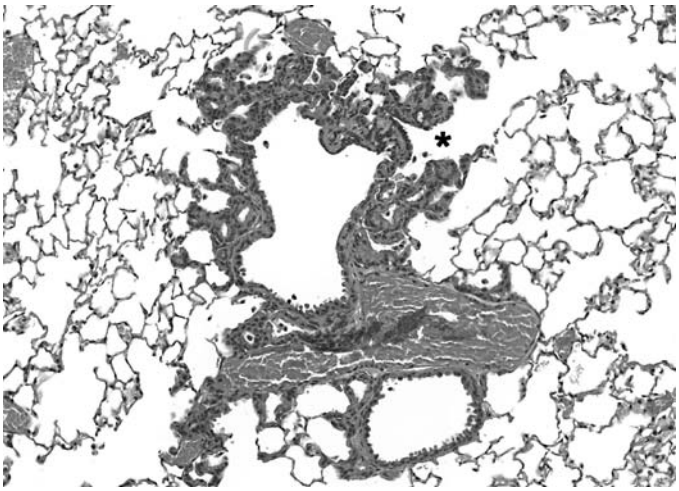


**PLATE 12**
Lung of a female B6C3F1 mouse exposed to 500 ppm cumene in the 2-year inhalation study. Note the peribronchiolar extension of metaplastic bronchiolar epithelium into adjacent alveoli (asterisk). H&E

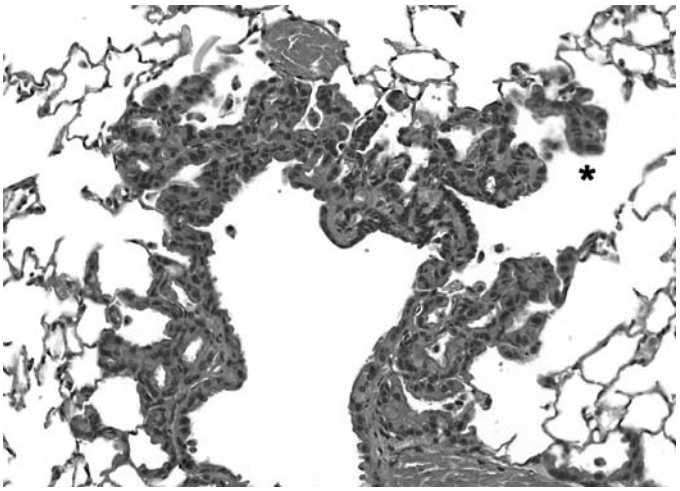


**PLATE 13**
Higher magnification of Plate 12. Note the metaplastic cuboidal bronchiolar cells extending into adjacent alveoli (asterisks). H&E

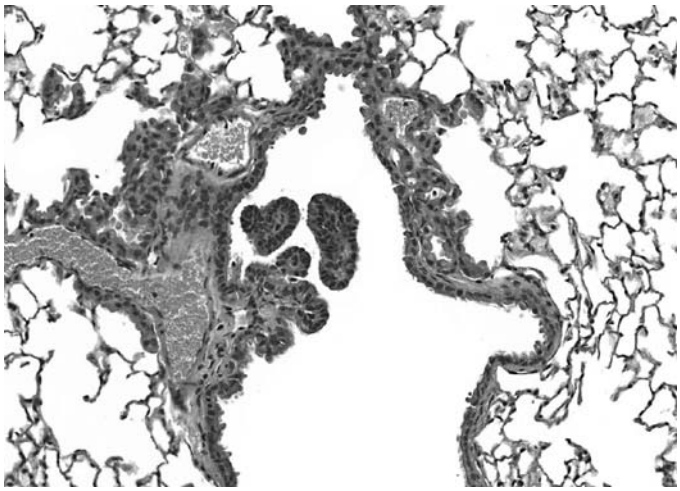


**PLATE 14**
Lung of a female B6C3F1 mouse exposed to 125 ppm cumene in the 2-year inhalation study. Small papillary projections were formed by the hyperplastic bronchiolar epithelium. H&E

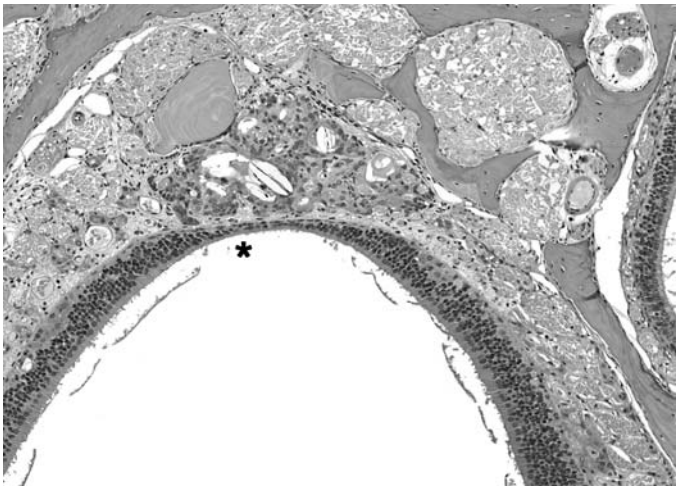


**PLATE 15**
Nasal tissues in Level III of a male B6C3F1 moused exposed to 1,000 ppm cumene in the 2-year inhalation study. Localized atrophic olfactory epithelium has pronounced thinning of the mucosa (asterisk). The adjacent mucosa has basal cell hyperplasia. Submucosal Bowman's glands are hyperplastic with granulomatous inflammation, lipoid cysts, and clefts. H&E

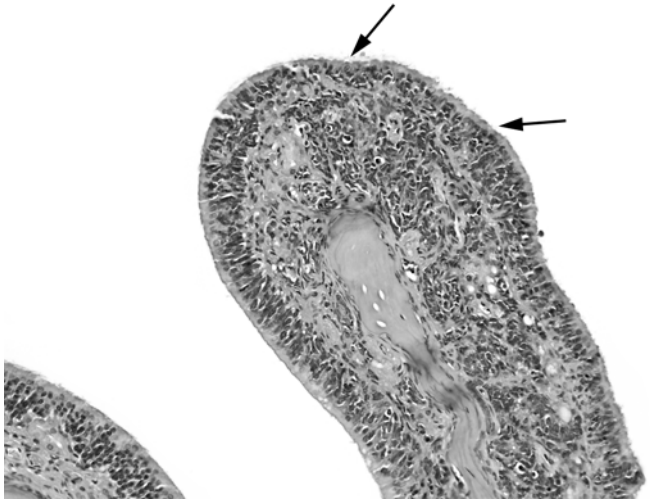


**PLATE 16**
Olfactory epithelium with atypical hyperplasia in nasal Level III of a male B6C3F1 mouse exposed to 1,000 ppm cumene in the 2-year inhalation study. Note the increased numbers of basophilic cells along the basement membrane resulting in disorganization of the olfactory epithelium and extension into the adjacent submucosal tissues (arrows). H&E

# DISCUSSION AND CONCLUSIONS

Cumene was nominated for study by the National Institute of Environmental Health Sciences because of its high production volume, presence in gasoline and other fuels, potential for human exposure, and lack of existing carcinogenicity test data. Cumene is a central nervous system depressant (Andrews and Snyder, 1993). At high exposure concentrations in the 2-week, 3-month, and 2-year rat and mouse studies, cumene induced ataxia, but surviving animals recovered and developed tolerance after a few days. Microscopic alterations in the peripheral and central nervous systems were not observed. Cushman *et al.* (1995) reported changes in the functional observational battery test in male and female rats 1 hour after a single 6-hour exposure to 1,200 ppm cumene; the changes included gait abnormalities, increased horizontal activity, decreased mean rectal temperature, and decreased toe-pinch withdrawal reflexes. The alterations in behavioral or motor activity disappeared within 24 hours. The findings were typical of those commonly observed following exposure to alkylbenzenes, such as toluene and ethylbenzene (Andrews and Snyder, 1993; Tegeris and Balster, 1994).

The highest exposure concentration selected for male and female rats and male mice in the 2-year studies was 1,000 ppm. Deaths, presumably from central nervous system depression, occurred at higher exposure concentrations in the 2-week studies, and deaths occurred at 1,000 ppm in the 3-month female mouse study, resulting in the selection of 500 ppm as the highest exposure concentration for the 2-year female mouse study.

In the 3-month rat study, the severities of renal cortical (proximal) tubular cell hyaline droplet accumulation and regeneration (hypertrophy, hyperplasia) in males increased with increasing exposure concentration. The incidences of medullary granular casts in the 250, 500, and 1,000 ppm male rats were significantly increased, and the severity of this lesion was generally exposure-concentration-related. Levels of $\alpha$2u-globulin in the kidney also were significantly greater in male rats exposed to 125 ppm or greater compared to the chamber controls. The absolute and relative kidney weights of exposed groups of male rats were increased. Relative kidney weights of female rats exposed to 250 ppm or greater were significantly increased, but no accompanying histopathology changes were observed. These changes were consistent with $\alpha$2u-globulin nephropathy.

In the 2-year study, cumene induced significantly increased incidences of olfactory epithelium basal cell hyperplasia in the nose for all exposed groups of rats and hyperplasia of the respiratory epithelium in all exposed groups of male rats and in 1,000 ppm females. Respiratory epithelium adenomas were observed in all exposed groups. A positive trend in the incidences of respiratory epithelium adenoma occurred in male rats but not in female rats. The incidences of adenoma in the 1,000 ppm males and females were lower than those seen at 500 ppm. However, if the incidences of respiratory epithelium hyperplasia and adenoma are considered together, exposure concentration-related increase trends are apparent. Hyperplasia of the respiratory epithelium and adenoma form a morphologic continuum. The areas of respiratory epithelial hyperplasia and adenomas were discrete foci with few or no abnormalities in the adjacent respiratory epithelium. Male rats appeared to be more sensitive than females to the progression from hyperplasia to adenoma caused by cumene. Progression of nasal respiratory epithelial adenomas to malignancy has been described in the literature (Brown *et al.*, 1991; Morgan and Harkema, 1996). No evidence of malignant progression was observed in the present studies. Hyperplasia of the respiratory epithelium in the nose was not observed in rats in the 3-month study nor in the 3-month or 2-year mouse studies.

In the 2-year rat study, incidences of renal tubule adenoma or carcinoma (combined) were increased in exposed males, and the increase was significant at 500 ppm. The renal tubular neoplasms were considered related to cumene exposure because spontaneous incidences of renal neoplasms in untreated male F344/N rats in inhalation studies are very low. Along with renal neoplasms, the incidences of renal papilla mineralization and renal pelvis transitional epithelium hyperplasia

were also increased in exposed male rats. Renal tubule hyperplasia was considered a preneoplastic lesion. The pathogenesis underlying the renal lesions in male rats in the 2-year study is likely similar to that observed with many structurally related chemicals, such as ethylbenzene. Cumene or a metabolite is thought to bind to $\alpha$2u-globulin causing the protein to resist lysosomal degradation. The pathogenesis process probably involves accumulation of protein droplets in the S2 segment of the proximal tubules, necrosis secondary to lysosomal overload, and increased cell proliferation. The continuing necrosis eventually overwhelms the capacity of the kidney to remove necrotic debris in tubular lumens, resulting in granular casts and mineralization of the papillary tubules. The histopathologic changes ultimately lead to the development of renal neoplasm. The pathogenesis of this syndrome has been widely discussed in the literature (Swenberg *et al.*, 1989; Montgomery and Seely, 1990; Melnick, 1992; Hard *et al.*, 1993; Goldstein and Schnellmann, 1996; Lehman-Mckeeman, 1997; Kohn and Melnick, 1999; Schnellmann, 2001). These changes were not seen in female rats, male or female mice, or humans.

In the 2-year mouse study, chemical-related neoplasms and nonneoplastic lesions in the lung included increased incidences in all exposed groups of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, alveolar/bronchiolar epithelium metaplasia, and bronchiole hyperplasia. The incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma or carcinoma (combined) in male and female mice exceeded the historical control range at all exposure concentrations, indicating an unequivocal carcinogenic effect. Alveolar/bronchiolar epithelial hyperplasia is considered a preneoplastic change and may progress to adenoma and then carcinoma (Foley *et al.*, 1991; Dixon *et al.*, 1999).

In the 2-year mouse study, cumene exposure induced olfactory epithelium lesions in the nose, including atrophy, basal cell hyperplasia, atypical hyperplasia, and gland hyperplasia, in males and females. Olfactory atypical basal cell hyperplasia may have represented a preneoplastic change. However, no nasal olfactory epithelium neoplasms were found in this study. A significant increase in the incidence of squamous metaplasia of the respiratory epithelium occurred in 500 ppm females.

In the 2-year mouse study, cumene exposure affected the livers of females to a greater extent than males. Exposure concentration-related increases in the incidences of hepatocellular adenoma or carcinoma (combined) were observed in female mice, and the increase in the 500 ppm group was significant. In males exposed to 500 or 1,000 ppm, the incidences of eosinophilic focus were increased. Ethylbenzene, which is structurally similar to cumene, also caused an increased incidence of hepatocellular neoplasms only in female mice (Chan *et al.*, 1998; NTP, 1999).

In the 2-year mouse study, increased incidences of forestomach epithelial hyperplasia and inflammation were observed in exposed males. The increases were probably related to irritating effects of cumene, and similar lesions are commonly seen in NTP inhalation studies, presumably resulting from ingestion of the chemical from fur or clearance from the lung through microciliary action.

Cumene is an alkylbenzene structurally similar to benzene, toluene, styrene, xylene, and ethylbenzene (Andrews and Snyder, 1993). Like other alkylbenzenes, cumene may be metabolized by oxidation of the side chain or by ring oxidation. Both biotransformations are catalyzed by cytochromes P450. However, a pathway for metabolic activation of cumene to an intermediate capable of reacting with protein or DNA is not obvious. There are no structural alerts. Cumene is a relatively small aromatic molecule, probably a substrate for CYP2E1 and CYP2F2. CYP2F2 has been located in Clara cells in mouse lung using immunohistochemical techniques. There was little immunological reaction for CYP2F in Clara cells from rat or hamster lungs. This observation correlates with rates of naphthalene biotransformation in the three species (Buckpitt *et al.*, 1995). The biotransformations of styrene have been extensively studied. While epoxidation of the vinyl group is the major biotransformation pathway, a ring-hydroxylated product, 4-vinylphenol, is also formed. Vinylphenol is more toxic than styrene oxide but requires further biotransformation to be toxic (Cruzan *et al.*, 2002). The phenol is more toxic to mouse lung than rat lung. The toxicity can be prevented by treatment with a CYP2F inhibitor, 5-phenyl-1-pentyne (Cruzan *et al.*, 2002). The observation that mice exhale more $^{14}CO_2$ than rats when dosed with ring-labeled [$^{14}$C]-styrene implies that the aromatic ring is broken and is consistent with more extensive metabolism in mice (Boogaard *et al.*, 2000). With this as a background, a pathway for activation of cumene can be proposed (Figure 6). Ring-hydroxylation to isopropylphenol is the proposed first step. There are at least three possible activated intermediates from

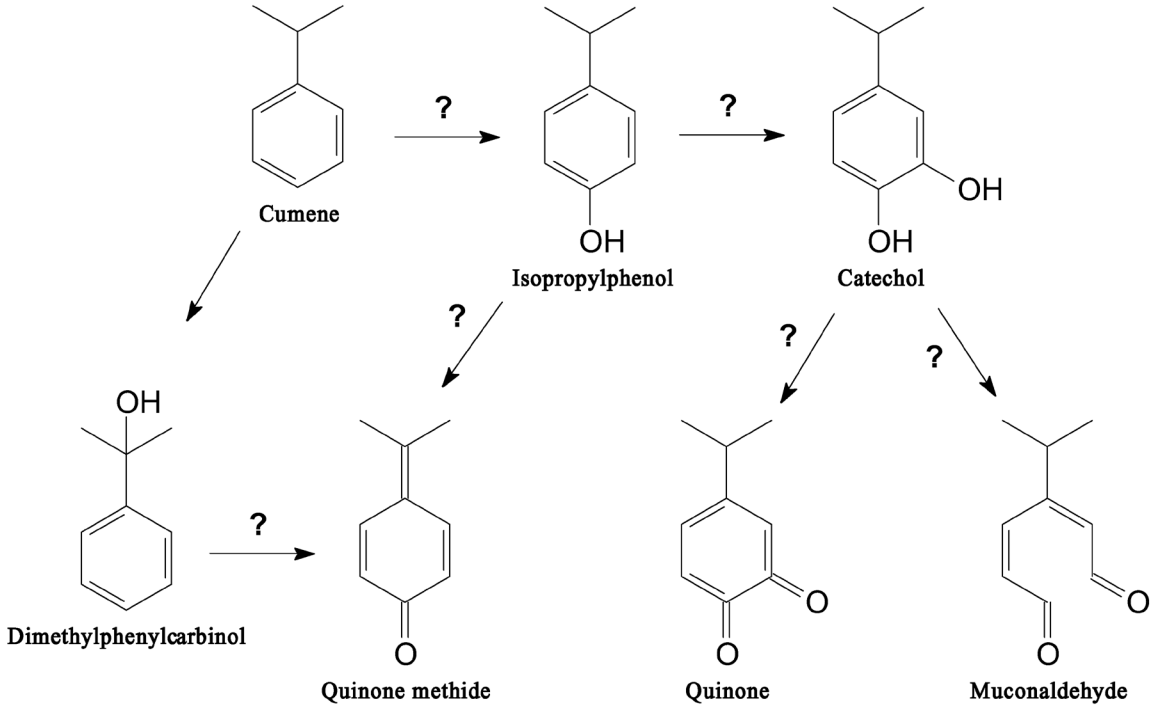



FIGURE 6
**Possible Reactive Metabolites of Cumene**

further biotransformation of the phenol: oxidation to a quinone methide, ring-opening to a muconaldehyde, or hydroxylation to a catechol and oxidation to a quinone. Biotransformation of cumene by CYP2F2 in mouse lung and nasal tissue and CYP2F4 in rat nasal tissue could lead to the observed species difference.

Studies of the absorption, distribution, metabolism, and excretion of cumene are ongoing and may provide more insight on the biotransformation and bioactivation. Preliminary results have confirmed the major urinary metabolites as described in Figure 6. Exhaled organic volatiles are composed of an approximately 1:1 mixture of cumene and α-methylstyrene. α-Methylstyrene has not previously been reported as a metabolite of cumene; however, the urinary metabolites 2-phenyl-1,2-propanediol and 2-hydroxyphenylpropanoic acid are likely to be derived from the epoxide of α-methylstyrene. Whether α-methylstyrene requires ring-hydroxylation as an activating step in producing reactive intermediates as described for styrene is not known; however, certain aspects of the toxicity/carcinogenicity of inhaled α-methylstyrene (NTP, 2007) are similar to those of cumene. For example, in rats, cumene at 1,000 ppm induced olfactory epithelial hyperplasia and respiratory epithelial neoplasms in males and females and α2u-globulin nephropathy and renal tubular neoplasms in males. α-Methylstyrene at 1,000 ppm also induced olfactory epithelial hyperplasia in males and females, although it did not appear to affect the respiratory epithelium, as did cumene, and caused α2u-globulin nephropathy and renal tubular neoplasms in males. In mice, cumene at 500 and 1,000 ppm induced olfactory epithelial hyperplasia and other nasal lesions in males and females, hepatocellular neoplasms in females, and hepatic eosinophilic foci in males. α-Methylstyrene at 600 ppm also induced olfactory epithelial hyperplasia in males and females and hepatocellular neoplasms in females. In contrast, the marked carcinogenic effects in the lung of male and female mice caused by cumene did not occur with α-methylstyrene. Thus, even though cumene can be metabolized to α-methylstyrene, the toxic and carcinogenic effects of cumene are more widespread than those of α-methylstyrene. Therefore, α-methylstyrene can not be the sole active contributor to the carcinogenicity of cumene. It is not known how α-methylstyrene interacts with cellular macromolecules in its carcinogenesis process, although a metabolic activation via styrene and styrene oxide has been suggested. There is no evidence that α-methylstyrene binds to DNA or protein. α-Methylstyrene is not mutagenic in *Salmonella* tests with or without S9. α-Methylstyrene did not induce micronuclei in erythrocytes in male mice, but in female mice, a significant increase in micronucleated normochromatic erythrocytes was seen at the highest concentration tested (NTP, 2007).

The present studies demonstrated that cumene is a multisite carcinogen both in mice, inducing neoplasms in the lung and liver, and in rats, inducing neoplasms in the nose and kidney. Multisite, multispecies carcinogens are frequently genotoxic carcinogens, but standard assays for genotoxicity did not detect cumene as a genotoxicant. Cumene was not mutagenic in the Ames test, and it did not induce micronuclei, indicative of chromosomal damage, in erythrocytes of mice exposed to cumene for 3 months by inhalation (Appendix E). Although positive results in the Ames assay and the 3-month mouse erythrocyte micronucleus assay are highly predictive of carcinogenicity, negative results are not predictive of noncarcinogenicity (Zeiger *et al.*, 1990; Witt *et al.*, 2000). Because of the extensive metabolism that cumene undergoes *in vivo*, it is possible that the proximate carcinogen is not generated in the *in vitro* Ames test, even with the addition of exogenous metabolic activation enzymes. Cumene did, however, induce a small, but statistically significant, increase in micronucleated erythrocytes in bone marrow of male rats treated by intraperitoneal injection. The contrast with the results in the 3-month micronucleus test in mice might be explained by differences in route and dosage between the two tests, such that greater exposure of the proerythrocyte target cells in the bone marrow was achieved in the rat study. The significantly increased frequencies of K-*ras* and *p53* mutations seen in lung neoplasms of mice exposed to cumene in this study (Appendix L) clearly imply genetic events related to cumene exposure, because these mutations were not observed in spontaneous neoplasms in the chamber control mice. Similar findings of mutations in cancer genes and negative results in *Salmonella* and mouse micronucleus tests have been observed in previous studies. For example, with the nongenotoxic agent oxazepam, mutations in the *B*-catenin gene were related to cytochrome p450 induction and oxidative stress. Also, chloroprene, which is inactive in *Salmonella* and mouse micronucleus studies, induced lung tumors with signature K-*ras* A to T transversions caused by adenine adducts related to a specific chloroprene metabolite.

## CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of cumene in male F344/N rats based on increased incidences of respiratory epithelial adenoma in the nose and renal tubule adenoma or carcinoma (combined). Increased incidences of interstitial cell adenoma of the testis may have been related to exposure to cumene. There was *some evidence of carcinogenic activity* of cumene in female F344/N rats based on the incidences of respiratory epithelium adenoma in the nose. There was *clear evidence of carcinogenic activity* of cumene in male B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. The increased incidences of hemangiosarcoma in the spleen and follicular cell adenoma in the thyroid gland in male mice may have been related to cumene exposure. There was *clear evidence of carcinogenic activity* of cumene in female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. Increased incidences of hepatocellular adenoma or carcinoma (combined) in female mice were also considered to be related to exposure to cumene.

Exposure of male rats to cumene resulted in nonneoplastic lesions of the kidney characteristic of $\alpha$2u-globulin accumulation. Exposure to cumene resulted in nonneoplastic lesions in the nose of male and female rats; the lung, nose, liver, and forestomach of male mice; and the lung and nose of female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Report 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 CUMENE

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 20 | 24 | 21 | 24 |
| Natural deaths | 4 | 3 | 2 | 2 |
| Survivors | | | | |
| Terminal sacrifice | 26 | 23 | 27 | 24 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Esophagus | (50) | (50) | (50) | (50) |
| Intestine large, cecum | (49) | (49) | (49) | (49) |
| Intestine large, colon | (49) | (50) | (50) | (50) |
| Intestine large, rectum | (49) | (50) | (50) | (50) |
| Intestine small, duodenum | (49) | (49) | (49) | (49) |
| Intestine small, ileum | (49) | (47) | (48) | (49) |
| Intestine small, jejunum | (48) | (47) | (48) | (49) |
| Liver | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, prostate | | | | 1 (2%) |
| Hepatocellular adenoma | | | 1 (2%) | |
| Hepatocellular carcinoma | | | | 2 (4%) |
| Mesentery | (7) | (13) | (10) | (6) |
| Carcinoma, metastatic, prostate | | | | 1 (17%) |
| Pancreas | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, prostate | | | | 1 (2%) |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Stomach, glandular | (50) | (50) | (50) | (50) |
| Tongue | (1) | (3) | (1) | (3) |
| Papilloma | | 1 (33%) | | |
| Tooth | | (1) | | |
| **Cardiovascular System** | | | | |
| Blood vessel | (1) | (1) | (1) | |
| Heart | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (2%) | 1 (2%) |
| Carcinoma, metastatic, prostate | | | | 1 (2%) |
| Pericardium, carcinoma, metastatic, lung | 1 (2%) | | | |
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (50) | (50) |
| Adenoma | | 2 (4%) | 3 (6%) | |
| Adrenal medulla | (50) | (50) | (50) | (50) |
| Pheochromocytoma benign | 7 (14%) | 6 (12%) | 9 (18%) | 8 (16%) |
| Pheochromocytoma malignant | 1 (2%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Bilateral, pheochromocytoma benign | | 1 (2%) | | 3 (6%) |
| Islets, pancreatic | (50) | (50) | (50) | (50) |
| Adenoma | 1 (2%) | 2 (4%) | 2 (4%) | 2 (4%) |
| Carcinoma | 2 (4%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Parathyroid gland | (45) | (49) | (49) | (48) |
| Pituitary gland | (50) | (50) | (49) | (50) |
| Adenoma | 36 (72%) | 38 (76%) | 25 (51%) | 30 (60%) |

**TABLE A1**
**Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Endocrine System** (continued) | | | | |
| Thyroid gland | (50) | (50) | (50) | (50) |
| C-cell, adenoma | 3 (6%) | 7 (14%) | 1 (2%) | 4 (8%) |
| C-cell, carcinoma | 1 (2%) | 2 (4%) | 1 (2%) | 2 (4%) |
| Follicular cell, adenoma | | 1 (2%) | | 1 (2%) |
| Follicular cell, carcinoma | 2 (4%) | | | 2 (4%) |
| **General Body System** | | | | |
| Peritoneum | (2) | (2) | (4) | (2) |
| Carcinoma, metastatic, prostate | | | | 1 (50%) |
| Carcinoma, metastatic, urinary bladder | | | 1 (25%) | |
| **Genital System** | | | | |
| Epididymis | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, prostate | | | | 1 (2%) |
| Preputial gland | (50) | (50) | (50) | (50) |
| Carcinoma | | 3 (6%) | 1 (2%) | |
| Prostate | (50) | (50) | (50) | (50) |
| Carcinoma | | | | 1 (2%) |
| Carcinoma, metastatic, urinary bladder | | | 1 (2%) | |
| Seminal vesicle | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, prostate | | | | 1 (2%) |
| Testes | (50) | (50) | (50) | (50) |
| Bilateral, interstitial cell, adenoma | 18 (36%) | 24 (48%) | 27 (54%) | 37 (74%) |
| Interstitial cell, adenoma | 18 (36%) | 14 (28%) | 13 (26%) | 9 (18%) |
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Lymph node | (7) | (10) | (8) | (14) |
| Deep cervical, carcinoma, metastatic, skin | | 1 (10%) | | |
| Lymph node, bronchial | (7) | (12) | (11) | (8) |
| Lymph node, mandibular | (1) | (3) | | (1) |
| Lymph node, mediastinal | (34) | (32) | (34) | (34) |
| Carcinoma, metastatic, thyroid gland | 1 (3%) | 1 (3%) | | |
| Lymph node, mesenteric | (50) | (50) | (50) | (50) |
| Spleen | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, prostate | | | | 1 (2%) |
| Thymus | (49) | (49) | (50) | (50) |
| **Integumentary System** | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Carcinoma | 1 (2%) | | 2 (4%) | |
| Carcinoma, multiple | 1 (2%) | | | |
| Fibroadenoma | | 1 (2%) | 3 (6%) | 2 (4%) |
| Skin | (50) | (50) | (50) | (50) |
| Basal cell adenoma | 1 (2%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Basal cell carcinoma | | | 2 (4%) | |
| Keratoacanthoma | 4 (8%) | 1 (2%) | 2 (4%) | 2 (4%) |
| Keratoacanthoma, multiple | | 1 (2%) | | 1 (2%) |
| Squamous cell papilloma | | | 1 (2%) | |
| Trichoepithelioma | | | | 1 (2%) |
| Sebaceous gland, adenoma | 1 (2%) | | | |

**TABLE A1**
**Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Integumentary System** (continued) | | | | |
| Skin (continued) | (50) | (50) | (50) | (50) |
| Sebaceous gland, carcinoma | | 1 (2%) | | |
| Subcutaneous tissue, carcinoma, metastatic, Harderian gland | | 1 (2%) | | |
| Subcutaneous tissue, fibroma | 3 (6%) | 3 (6%) | 3 (6%) | 4 (8%) |
| Subcutaneous tissue, fibrosarcoma | | | | 1 (2%) |
| Subcutaneous tissue, lipoma | | 1 (2%) | 1 (2%) | |
| Subcutaneous tissue, schwannoma, benign | | | | 1 (2%) |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Cranium, carcinoma, metastatic, Zymbal's gland | 1 (2%) | | | |
| Skeletal muscle | (1) | (2) | (2) | (1) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (50%) | |
| Carcinoma, metastatic, lung | 1 (100%) | | | |
| Carcinoma, metastatic, prostate | | | | 1 (100%) |
| Carcinoma, metastatic, urinary bladder | | | 1 (50%) | |
| Rhabdomyosarcoma | | 1 (50%) | | |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Granular cell tumor malignant | | 1 (2%) | | |
| **Respiratory System** | | | | |
| Larynx | (50) | (50) | (50) | (50) |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | 1 (2%) | 2 (4%) | 2 (4%) | 2 (4%) |
| Alveolar/bronchiolar carcinoma | | | | 2 (4%) |
| Alveolar/bronchiolar carcinoma, multiple | 1 (2%) | | 1 (2%) | 1 (2%) |
| Carcinoma, metastatic, prostate | | | | 1 (2%) |
| Carcinoma, metastatic, skin | | 1 (2%) | 1 (2%) | |
| Carcinoma, metastatic, thyroid gland | | | | 1 (2%) |
| Carcinoma, metastatic, urinary bladder | | | 1 (2%) | |
| Pheochromocytoma malignant, metastatic, adrenal medulla | 1 (2%) | | | 1 (2%) |
| Nose | (50) | (50) | (49) | (50) |
| Glands, respiratory epithelium, adenoma | | | 1 (2%) | |
| Respiratory epithelium, adenoma | | 6 (12%) | 15 (31%) | 4 (8%) |
| Respiratory epithelium, adenoma, multiple | | 1 (2%) | 2 (4%) | 6 (12%) |
| Pleura | (5) | (3) | (5) | (6) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (20%) | |
| Trachea | (50) | (50) | (50) | (50) |
| **Special Senses System** | | | | |
| Eye | (50) | (50) | (49) | (50) |
| Carcinoma, metastatic, Harderian gland | | 1 (2%) | | |
| Harderian gland | (50) | (50) | (50) | (50) |
| Carcinoma | | 1 (2%) | | |
| Zymbal's gland | (1) | | | (2) |
| Carcinoma | 1 (100%) | | | 2 (100%) |

**TABLE A1**
**Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, prostate | | | | 1 (2%) |
| Lipoma | 1 (2%) | | | 1 (2%) |
| Bilateral, renal tubule, carcinoma | | | 1 (2%) | |
| Renal tubule, adenoma | 1 (2%) | 4 (8%) | 5 (10%) | 4 (8%) |
| Renal tubule, carcinoma | 1 (2%) | 1 (2%) | 2 (4%) | 3 (6%) |
| Ureter | | (1) | | (1) |
| Carcinoma, metastatic, prostate | | | | 1 (100%) |
| Urethra | | | (1) | (1) |
| Carcinoma, metastatic, prostate | | | | 1 (100%) |
| Urinary bladder | (50) | (50) | (49) | (50) |
| Transitional epithelium, carcinoma | | | 2 (4%) | 1 (2%) |
| Transitional epithelium, papilloma | | | | 2 (4%) |
| **Systemic Lesions** | | | | |
| Multiple organs[b] | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | | | | 1 (2%) |
| Leukemia mononuclear | 26 (52%) | 32 (64%) | 31 (62%) | 28 (56%) |
| Lymphoma malignant | | | | 1 (2%) |
| Mesothelioma malignant | 3 (6%) | 2 (4%) | 3 (6%) | 1 (2%) |
| **Neoplasm Summary** | | | | |
| Total animals with primary neoplasms[c] | 50 | 50 | 50 | 50 |
| Total primary neoplasms | 135 | 163 | 166 | 176 |
| Total animals with benign neoplasms | 48 | 48 | 47 | 50 |
| Total benign neoplasms | 95 | 117 | 117 | 125 |
| Total animals with malignant neoplasms | 36 | 37 | 38 | 39 |
| Total malignant neoplasms | 40 | 46 | 49 | 51 |
| Total animals with metastatic neoplasms | 4 | 4 | 3 | 4 |
| Total metastatic neoplasms | 5 | 6 | 8 | 16 |

[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 A2**
**Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Adrenal Cortex: Adenoma** | | | | |
| Overall rate[a] | 0/50 (0%) | 2/50 (4%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted rate[b] | 0.0% | 5.1% | 7.3% | 0.0% |
| Terminal rate[c] | 0/26 (0%) | 1/23 (4%) | 2/27 (7%) | 0/24 (0%) |
| First incidence (days) | —[e] | 665 | 626 | —[f] |
| Poly-3 test[d] | P=0.519N | P=0.226 | P=0.117 | — |
| **Adrenal Medulla: Benign Pheochromocytoma** | | | | |
| Overall rate | 7/50 (14%) | 7/50 (14%) | 9/50 (18%) | 11/50 (22%) |
| Adjusted rate | 16.7% | 17.4% | 21.5% | 25.9% |
| Terminal rate | 5/26 (19%) | 4/23 (17%) | 6/27 (22%) | 8/24 (33%) |
| First incidence (days) | 647 | 624 | 600 | 688 |
| Poly-3 test | P=0.149 | P=0.583 | P=0.387 | P=0.221 |
| **Adrenal Medulla: Benign or Malignant Pheochromocytoma** | | | | |
| Overall rate | 7/50 (14%) | 7/50 (14%) | 10/50 (20%) | 13/50 (26%) |
| Adjusted rate | 16.7% | 17.4% | 23.8% | 30.5% |
| Terminal rate | 5/26 (19%) | 4/23 (17%) | 6/27 (22%) | 9/24 (38%) |
| First incidence (days) | 647 | 624 | 600 | 688 |
| Poly-3 test | P=0.054 | P=0.583 | P=0.293 | P=0.103 |
| **Kidney (Renal Tubule): Adenoma** | | | | |
| Overall rate | 1/50 (2%) | 4/50 (8%) | 5/50 (10%) | 4/50 (8%) |
| Adjusted rate | 2.4% | 10.0% | 12.1% | 9.3% |
| Terminal rate | 1/26 (4%) | 1/23 (4%) | 2/27 (7%) | 2/24 (8%) |
| First incidence (days) | 729 (T) | 665 | 679 | 635 |
| Poly-3 test | P=0.219 | P=0.165 | P=0.099 | P=0.187 |
| **Kidney (Renal Tubule): Carcinoma** | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 3/50 (6%) | 3/50 (6%) |
| Adjusted rate | 2.4% | 2.5% | 7.3% | 7.0% |
| Terminal rate | 1/26 (4%) | 1/23 (4%) | 2/27 (7%) | 2/24 (8%) |
| First incidence (days) | 729 (T) | 729 (T) | 639 | 618 |
| Poly-3 test | P=0.180 | P=0.749 | P=0.302 | P=0.314 |
| **Kidney (Renal Tubule): Adenoma or Carcinoma** | | | | |
| Overall rate | 2/50 (4%) | 5/50 (10%) | 8/50 (16%) | 7/50 (14%) |
| Adjusted rate | 4.8% | 12.5% | 19.2% | 16.2% |
| Terminal rate | 2/26 (8%) | 2/23 (9%) | 4/27 (15%) | 4/24 (17%) |
| First incidence (days) | 729 (T) | 665 | 639 | 618 |
| Poly-3 test | P=0.087 | P=0.198 | P=0.044 | P=0.087 |
| **Lung: Alveolar/bronchiolar Carcinoma** | | | | |
| Overall rate | 1/50 (2%) | 0/50 (0%) | 1/50 (2%) | 3/50 (6%) |
| Adjusted rate | 2.4% | 0.0% | 2.4% | 7.0% |
| Terminal rate | 1/26 (4%) | 0/23 (0%) | 1/27 (4%) | 1/24 (4%) |
| First incidence (days) | 729 (T) | — | 729 (T) | 674 |
| Poly-3 test | P=0.104 | P=0.511N | P=0.758 | P=0.314 |
| **Lung: Alveolar/bronchiolar Adenoma or Carcinoma** | | | | |
| Overall rate | 2/50 (4%) | 2/50 (4%) | 3/50 (6%) | 5/50 (10%) |
| Adjusted rate | 4.8% | 5.1% | 7.3% | 11.7% |
| Terminal rate | 2/26 (8%) | 2/23 (9%) | 2/27 (7%) | 3/24 (13%) |
| First incidence (days) | 729 (T) | 729 (T) | 670 | 674 |
| Poly-3 test | P=0.125 | P=0.675 | P=0.496 | P=0.226 |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Mammary Gland: Fibroadenoma** | | | | |
| Overall rate | 0/50 (0%) | 1/50 (2%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate | 0.0% | 2.5% | 7.3% | 4.7% |
| Terminal rate | 0/26 (0%) | 1/23 (4%) | 3/27 (11%) | 2/24 (8%) |
| First incidence (days) | — | 729 (T) | 729 (T) | 729 (T) |
| Poly-3 test | P=0.174 | P=0.489 | P=0.115 | P=0.241 |
| **Mammary Gland: Fibroadenoma or Carcinoma** | | | | |
| Overall rate | 2/50 (4%) | 1/50 (2%) | 5/50 (10%) | 2/50 (4%) |
| Adjusted rate | 4.8% | 2.5% | 12.2% | 4.7% |
| Terminal rate | 1/26 (4%) | 1/23 (4%) | 5/27 (19%) | 2/24 (8%) |
| First incidence (days) | 628 | 729 (T) | 729 (T) | 729 (T) |
| Poly-3 test | P=0.479 | P=0.522N | P=0.205 | P=0.691N |
| **Nose: Adenoma** | | | | |
| Overall rate | 0/50 (0%) | 7/50 (14%) | 18/49 (37%) | 10/50 (20%) |
| Adjusted rate | 0.0% | 17.6% | 43.2% | 23.3% |
| Terminal rate | 0/26 (0%) | 5/23 (22%) | 13/27 (48%) | 7/24 (29%) |
| First incidence (days) | — | 639 | 638 | 674 |
| Poly-3 test | P=0.004 | P=0.006 | P<0.001 | P<0.001 |
| **Pancreatic Islets: Adenoma or Carcinoma** | | | | |
| Overall rate | 3/50 (6%) | 3/50 (6%) | 4/50 (8%) | 3/50 (6%) |
| Adjusted rate | 7.2% | 7.6% | 9.7% | 7.1% |
| Terminal rate | 2/26 (8%) | 3/23 (13%) | 3/27 (11%) | 3/24 (13%) |
| First incidence (days) | 689 | 729 (T) | 679 | 729 (T) |
| Poly-3 test | P=0.578 | P=0.636 | P=0.492 | P=0.657N |
| **Pituitary Gland (Pars Distalis): Adenoma** | | | | |
| Overall rate | 36/50 (72%) | 38/50 (76%) | 25/49 (51%) | 30/50 (60%) |
| Adjusted rate | 76.2% | 80.4% | 58.4% | 62.9% |
| Terminal rate | 20/26 (77%) | 18/23 (78%) | 17/27 (63%) | 13/24 (54%) |
| First incidence (days) | 296 | 480 | 625 | 453 |
| Poly-3 test | P=0.030N | P=0.396 | P=0.048N | P=0.110N |
| **Preputial Gland: Carcinoma** | | | | |
| Overall rate | 0/50 (0%) | 3/50 (6%) | 1/50 (2%) | 0/50 (0%) |
| Adjusted rate | 0.0% | 7.5% | 2.4% | 0.0% |
| Terminal rate | 0/26 (0%) | 1/23 (4%) | 1/27 (4%) | 0/24 (0%) |
| First incidence (days) | — | 639 | 729 (T) | — |
| Poly-3 test | P=0.363N | P=0.111 | P=0.497 | — |
| **Preputial Gland: Adenoma or Carcinoma** | | | | |
| Overall rate | 0/50 (0%) | 3/50 (6%) | 1/50 (2%) | 0/50 (0%) |
| Adjusted rate | 0.0% | 7.5% | 2.4% | 0.0% |
| Terminal rate | 0/26 (0%) | 1/23 (4%) | 1/27 (4%) | 0/24 (0%) |
| First incidence (days) | — | 639 | 729 (T) | — |
| Poly-3 test | P=0.363N | P=0.111 | P=0.497 | — |
| **Skin: Keratoacanthoma** | | | | |
| Overall rate | 4/50 (8%) | 2/50 (4%) | 2/50 (4%) | 3/50 (6%) |
| Adjusted rate | 9.5% | 5.1% | 4.9% | 7.1% |
| Terminal rate | 1/26 (4%) | 2/23 (9%) | 2/27 (7%) | 1/24 (4%) |
| First incidence (days) | 613 | 729 (T) | 729 (T) | 677 |
| Poly-3 test | P=0.464N | P=0.369N | P=0.350N | P=0.496N |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Skin: Squamous Cell Papilloma or Keratoacanthoma** | | | | |
| Overall rate | 4/50 (8%) | 2/50 (4%) | 3/50 (6%) | 3/50 (6%) |
| Adjusted rate | 9.5% | 5.1% | 7.3% | 7.1% |
| Terminal rate | 1/26 (4%) | 2/23 (9%) | 3/27 (11%) | 1/24 (4%) |
| First incidence (days) | 613 | 729 (T) | 729 (T) | 677 |
| Poly-3 test | P=0.482N | P=0.369N | P=0.516N | P=0.496N |
| **Skin: Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma** | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate | 2.4% | 2.5% | 7.2% | 4.7% |
| Terminal rate | 1/26 (4%) | 1/23 (4%) | 1/27 (4%) | 2/24 (8%) |
| First incidence (days) | 729 (T) | 729 (T) | 554 | 729 (T) |
| Poly-3 test | P=0.342 | P=0.749 | P=0.305 | P=0.506 |
| **Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma** | | | | |
| Overall rate | 5/50 (10%) | 3/50 (6%) | 6/50 (12%) | 5/50 (10%) |
| Adjusted rate | 11.8% | 7.6% | 14.4% | 11.8% |
| Terminal rate | 2/26 (8%) | 3/23 (13%) | 4/27 (15%) | 3/24 (13%) |
| First incidence (days) | 613 | 729 (T) | 554 | 677 |
| Poly-3 test | P=0.478 | P=0.394N | P=0.491 | P=0.626N |
| **Skin: Fibroma** | | | | |
| Overall rate | 3/50 (6%) | 3/50 (6%) | 3/50 (6%) | 4/50 (8%) |
| Adjusted rate | 7.2% | 7.6% | 7.3% | 9.4% |
| Terminal rate | 2/26 (8%) | 2/23 (9%) | 2/27 (7%) | 2/24 (8%) |
| First incidence (days) | 632 | 711 | 688 | 677 |
| Poly-3 test | P=0.423 | P=0.635 | P=0.654 | P=0.508 |
| **Skin: Fibroma or Fibrosarcoma** | | | | |
| Overall rate | 3/50 (6%) | 3/50 (6%) | 3/50 (6%) | 5/50 (10%) |
| Adjusted rate | 7.2% | 7.6% | 7.3% | 11.7% |
| Terminal rate | 2/26 (8%) | 2/23 (9%) | 2/27 (7%) | 3/24 (13%) |
| First incidence (days) | 632 | 711 | 688 | 677 |
| Poly-3 test | P=0.271 | P=0.635 | P=0.654 | P=0.364 |
| **Testes: Adenoma** | | | | |
| Overall rate | 36/50 (72%) | 38/50 (76%) | 40/50 (80%) | 46/50 (92%) |
| Adjusted rate | 80.0% | 84.6% | 85.7% | 96.1% |
| Terminal rate | 24/26 (92%) | 22/23 (96%) | 25/27 (93%) | 24/24 (100%) |
| First incidence (days) | 558 | 536 | 460 | 541 |
| Poly-3 test | P=0.006 | P=0.370 | P=0.311 | P=0.007 |
| **Thyroid Gland (C-Cell): Adenoma** | | | | |
| Overall rate | 3/50 (6%) | 7/50 (14%) | 1/50 (2%) | 4/50 (8%) |
| Adjusted rate | 7.1% | 17.4% | 2.4% | 9.4% |
| Terminal rate | 1/26 (4%) | 3/23 (13%) | 0/27 (0%) | 3/24 (13%) |
| First incidence (days) | 571 | 589 | 702 | 677 |
| Poly-3 test | P=0.485N | P=0.133 | P=0.318N | P=0.500 |
| **Thyroid Gland (C-Cell): Adenoma or Carcinoma** | | | | |
| Overall rate | 3/50 (6%) | 9/50 (18%) | 2/50 (4%) | 6/50 (12%) |
| Adjusted rate | 7.1% | 22.0% | 4.9% | 14.1% |
| Terminal rate | 1/26 (4%) | 4/23 (17%) | 1/27 (4%) | 5/24 (21%) |
| First incidence (days) | 571 | 541 | 702 | 677 |
| Poly-3 test | P=0.432 | P=0.048 | P=0.516N | P=0.240 |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Thyroid Gland (Follicular Cell): Adenoma or Carcinoma** | | | | |
| Overall rate | 2/50 (4%) | 1/50 (2%) | 0/50 (0%) | 3/50 (6%) |
| Adjusted rate | 4.8% | 2.5% | 0.0% | 7.0% |
| Terminal rate | 2/26 (8%) | 0/23 (0%) | 0/27 (0%) | 1/24 (4%) |
| First incidence (days) | 729 (T) | 686 | — | 677 |
| Poly-3 test | P=0.348 | P=0.518N | P=0.240N | P=0.511 |
| **Urinary Bladder: Papilloma or Carcinoma** | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 2/49 (4%) | 3/50 (6%) |
| Adjusted rate | 0.0% | 0.0% | 4.8% | 7.1% |
| Terminal rate | 0/26 (0%) | 0/23 (0%) | 0/27 (0%) | 2/24 (8%) |
| First incidence (days) | — | — | 213 | 677 |
| Poly-3 test | P=0.030 | — | P=0.238 | P=0.122 |
| **All Organs: Mononuclear Leukemia** | | | | |
| Overall rate | 26/50 (52%) | 32/50 (64%) | 31/50 (62%) | 28/50 (56%) |
| Adjusted rate | 57.8% | 69.7% | 65.5% | 60.0% |
| Terminal rate | 12/26 (46%) | 14/23 (61%) | 13/27 (48%) | 11/24 (46%) |
| First incidence (days) | 600 | 519 | 460 | 515 |
| Poly-3 test | P=0.490N | P=0.160 | P=0.289 | P=0.501 |
| **All Organs: Malignant Mesothelioma** | | | | |
| Overall rate | 3/50 (6%) | 2/50 (4%) | 3/50 (6%) | 1/50 (2%) |
| Adjusted rate | 7.1% | 5.0% | 7.2% | 2.4% |
| Terminal rate | 2/26 (8%) | 0/23 (0%) | 1/27 (4%) | 1/24 (4%) |
| First incidence (days) | 599 | 575 | 634 | 729 (T) |
| Poly-3 test | P=0.253N | P=0.522N | P=0.659 | P=0.302N |
| **All Organs: Benign Neoplasms** | | | | |
| Overall rate | 48/50 (96%) | 48/50 (96%) | 47/50 (94%) | 50/50 (100%) |
| Adjusted rate | 97.5% | 98.8% | 97.5% | 100% |
| Terminal rate | 26/26 (100%) | 23/23 (100%) | 27/27 (100%) | 24/24 (100%) |
| First incidence (days) | 296 | 480 | 460 | 453 |
| Poly-3 test | P=0.261 | P=0.633 | P=0.770 | P=0.400 |
| **All Organs: Malignant Neoplasms** | | | | |
| Overall rate | 36/50 (72%) | 37/50 (74%) | 38/50 (76%) | 39/50 (78%) |
| Adjusted rate | 77.5% | 78.1% | 77.3% | 80.4% |
| Terminal rate | 19/26 (73%) | 16/23 (70%) | 17/27 (63%) | 17/24 (71%) |
| First incidence (days) | 495 | 445 | 213 | 515 |
| Poly-3 test | P=0.410 | P=0.576 | P=0.588N | P=0.462 |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **All Organs: Benign or Malignant Neoplasms** | | | | |
| Overall rate | 50/50 (100%) | 50/50 (100%) | 50/50 (100%) | 50/50 (100%) |
| Adjusted rate | 100% | 100% | 100% | 100% |
| Terminal rate | 26/26 (100%) | 23/23 (100%) | 27/27 (100%) | 24/24 (100%) |
| First incidence (days) | 296 | 445 | 213 | 453 |
| Poly-3 test | — | — | — | — |

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, kidney, lung, nose, pancreatic islets, pituitary gland, preputial gland, testes, thyroid gland, and urinary bladder; 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 are the P values 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 Control 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 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 2, 2007

**TABLE A3b**
**Historical Incidence of Kidney Neoplasms in Control Male F344/N Rats**[a]

| Study | Incidence in Controls | | | |
|---|---|---|---|---|
| | Lipoma | Renal Tubule Adenoma | Renal Tubule Carcinoma | Renal Tubule Adenoma or Carcinoma |
| **Historical Incidence: Inhalation Studies** | | | | |
| Cumene | 1/50 | 1/50 | 1/50 | 2/50 |
| Decalin | 0/50 | 1/50 | 0/50 | 1/50 |
| Divinylbenzene | 0/50 | 0/50 | 0/50 | 0/50 |
| Methyl isobutyl ketone | 0/50 | 0/50 | 0/50 | 0/50 |
| α-Methylstyrene | 0/50 | 0/50 | 0/50 | 0/50 |
| Propargyl alcohol | 0/49 | 0/49 | 0/49 | 0/49 |
| Propylene glycol mono-*t*-butyl ether | 0/50 | 1/50 | 0/50 | 1/50 |
| Stoddard solvent IIC | 0/50 | 0/50 | 1/50 | 1/50 |
| Vanadium pentoxide | 0/50 | 1/50 | 0/50 | 1/50 |
| **Overall Historical Incidence: Inhalation Studies** | | | | |
| Total (%) | 1/449 (0.2%) | 4/449 (0.9%) | 2/449 (0.5%) | 6/449 (1.3%) |
| Mean ± standard deviation | 0.2% ± 0.7% | 0.9% ± 1.0% | 0.4% ± 0.9% | 1.3% ± 1.4% |
| Range | 0%-2% | 0%-2% | 0%-2% | 0%-4% |
| **Overall Historical Incidence: All Routes** | | | | |
| Total (%) | 2/1,436 (0.1%) | 8/1,436 (0.6%) | 2/1,436 (0.1%) | 10/1,436 (0.7%) |
| Mean ± standard deviation | 0.1% ± 0.5% | 0.6% ± 0.8% | 0.1% ± 0.5% | 0.7% ± 1.0% |
| Range | 0%-2% | 0%-2% | 0%-2% | 0%-4% |

[a] Data as of March 2, 2007

**TABLE A3c**
**Historical Incidence of Adenoma of the Testis in Control Male F344/N Rats[a]**

| Study | Incidence in Controls |
|---|---|
| **Historical Incidence: Inhalation Studies** | |
| Cumene | 36/50 |
| Decalin | 40/50 |
| Divinylbenzene | 38/50 |
| Methyl isobutyl ketone | 42/50 |
| α-Methylstyrene | 33/50 |
| Propargyl alcohol | 38/49 |
| Propylene glycol mono-*t*-butyl ether | 41/50 |
| Stoddard solvent IIC | 41/50 |
| Vanadium pentoxide | 36/50 |
| **Overall Historical Incidence: Inhalation Studies** | |
| Total (%) | 345/449 (76.8%) |
| Mean ± standard deviation | 76.8% ± 5.9% |
| Range | 66%-84% |
| **Overall Historical Incidence: All Routes** | |
| Total (%) | 1,242/1,449 (85.7%) |
| Mean ± standard deviation | 85.5% ± 8.7% |
| Range | 66%-98% |

[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 Cumene[a]**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 20 | 24 | 21 | 24 |
| Natural deaths | 4 | 3 | 2 | 2 |
| Survivors | | | | |
| Terminal sacrifice | 26 | 23 | 27 | 24 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Esophagus | (50) | (50) | (50) | (50) |
| Foreign body | | 1 (2%) | | |
| Intestine large, cecum | (49) | (49) | (49) | (49) |
| Intestine large, colon | (49) | (50) | (50) | (50) |
| Diverticulum | 1 (2%) | | | |
| Intestine large, rectum | (49) | (50) | (50) | (50) |
| Intestine small, duodenum | (49) | (49) | (49) | (49) |
| Intestine small, ileum | (49) | (47) | (48) | (49) |
| Intestine small, jejunum | (48) | (47) | (48) | (49) |
| Inflammation, chronic active | 1 (2%) | | | |
| Thrombosis | 1 (2%) | | | |
| Ulcer | 1 (2%) | | | |
| Liver | (50) | (50) | (50) | (50) |
| Angiectasis | | 1 (2%) | | |
| Basophilic focus | 7 (14%) | 4 (8%) | 8 (16%) | 6 (12%) |
| Clear cell focus | 12 (24%) | 6 (12%) | 11 (22%) | 11 (22%) |
| Degeneration, cystic | 3 (6%) | 1 (2%) | 4 (8%) | 6 (12%) |
| Eosinophilic focus | | 1 (2%) | 3 (6%) | |
| Eosinophilic focus, multiple | | | 1 (2%) | |
| Fatty change, diffuse | | 1 (2%) | | |
| Hepatodiaphragmatic nodule | 3 (6%) | 8 (16%) | 2 (4%) | 3 (6%) |
| Inflammation, suppurative | | 1 (2%) | | |
| Mixed cell focus | 1 (2%) | | 3 (6%) | 1 (2%) |
| Necrosis | 3 (6%) | 3 (6%) | 3 (6%) | 3 (6%) |
| Vacuolization cytoplasmic | 6 (12%) | 2 (4%) | 3 (6%) | 2 (4%) |
| Bile duct, hyperplasia | 7 (14%) | 4 (8%) | 12 (24%) | 7 (14%) |
| Hepatocyte, regeneration | 1 (2%) | | | |
| Oval cell, hyperplasia | | | | 1 (2%) |
| Periportal, inflammation, chronic | | | 2 (4%) | |
| Mesentery | (7) | (13) | (10) | (6) |
| Hemorrhage | | 1 (8%) | | 1 (17%) |
| Necrosis | 6 (86%) | 10 (77%) | 10 (100%) | 3 (50%) |
| Fat, hemorrhage | 1 (14%) | | | 1 (17%) |
| Fat, necrosis | | 1 (8%) | | |
| Pancreas | (50) | (50) | (50) | (50) |
| Hyperplasia | | | | 1 (2%) |
| Vacuolization cytoplasmic | | 1 (2%) | | |
| Acinus, atrophy | 2 (4%) | 2 (4%) | 2 (4%) | 2 (4%) |
| Acinus, hyperplasia | | | 1 (2%) | 3 (6%) |

[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 Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Alimentary System** (continued) | | | | |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | | | |
| Inflammation, suppurative | | | 2 (4%) | |
| Necrosis | 1 (2%) | | | |
| Ulcer | 3 (6%) | 7 (14%) | 1 (2%) | 8 (16%) |
| Epithelium, hyperplasia | 6 (12%) | 11 (22%) | 7 (14%) | 10 (20%) |
| Stomach, glandular | (50) | (50) | (50) | (50) |
| Erosion | 2 (4%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Hyperplasia, lymphoid | | | 1 (2%) | |
| Mineralization | 1 (2%) | | | |
| Ulcer | 3 (6%) | | 1 (2%) | 1 (2%) |
| Tongue | (1) | (3) | (1) | (3) |
| Epithelium, hyperplasia | 1 (100%) | 2 (67%) | 1 (100%) | 3 (100%) |
| Tooth | | (1) | | |
| Inflammation | | 1 (100%) | | |
| **Cardiovascular System** | | | | |
| Blood vessel | (1) | (1) | (1) | |
| Mineralization | 1 (100%) | | | |
| Heart | (50) | (50) | (50) | (50) |
| Cardiomyopathy | 9 (18%) | 7 (14%) | 12 (24%) | 11 (22%) |
| Atrium, thrombosis | 3 (6%) | 6 (12%) | 4 (8%) | 4 (8%) |
| Valve, cardiomyopathy | 1 (2%) | | | |
| Valve, hemorrhage | | | 1 (2%) | |
| Valve, thrombosis | 1 (2%) | | | |
| Ventricle, hypertrophy | | | | 1 (2%) |
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (50) | (50) |
| Hyperplasia | 17 (34%) | 16 (32%) | 10 (20%) | 10 (20%) |
| Hypertrophy | | | 1 (2%) | |
| Vacuolization cytoplasmic | 8 (16%) | 5 (10%) | 7 (14%) | 4 (8%) |
| Adrenal medulla | (50) | (50) | (50) | (50) |
| Hyperplasia | 11 (22%) | 13 (26%) | 18 (36%) | 12 (24%) |
| Bilateral, hyperplasia | | | | 1 (2%) |
| Islets, pancreatic | (50) | (50) | (50) | (50) |
| Hyperplasia | 1 (2%) | 1 (2%) | | |
| Parathyroid gland | (45) | (49) | (49) | (48) |
| Hyperplasia | 1 (2%) | 1 (2%) | | |
| Pituitary gland | (50) | (50) | (49) | (50) |
| Cyst | | 1 (2%) | 2 (4%) | |
| Hemorrhage | 1 (2%) | 1 (2%) | 1 (2%) | 3 (6%) |
| Hyperplasia | 6 (12%) | 7 (14%) | 13 (27%) | 6 (12%) |
| Pars intermedia, hyperplasia | 1 (2%) | | | |
| Thyroid gland | (50) | (50) | (50) | (50) |
| C-cell, hyperplasia | 7 (14%) | 11 (22%) | 6 (12%) | 8 (16%) |
| Follicular cell, cyst | | 1 (2%) | | |
| Follicular cell, hyperplasia | | | 1 (2%) | |
| **General Body System** | | | | |
| Peritoneum | (2) | (2) | (4) | (2) |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Genital System** | | | | |
| Epididymis | (50) | (50) | (50) | (50) |
| Preputial gland | (50) | (50) | (50) | (50) |
| Cyst | 1 (2%) | | 2 (4%) | |
| Hyperplasia | | | | 1 (2%) |
| Inflammation, suppurative | | | 1 (2%) | |
| Prostate | (50) | (50) | (50) | (50) |
| Hyperplasia | | 1 (2%) | 1 (2%) | 2 (4%) |
| Inflammation, suppurative | 42 (84%) | 32 (64%) | 27 (54%) | 28 (56%) |
| Seminal vesicle | (50) | (50) | (50) | (50) |
| Dilatation | | 1 (2%) | | |
| Hyperplasia | | 1 (2%) | | |
| Testes | (50) | (50) | (50) | (50) |
| Necrosis | | | 1 (2%) | |
| Artery, inflammation, chronic active | | 1 (2%) | 2 (4%) | |
| Bilateral, interstitial cell, hyperplasia | | | | 1 (2%) |
| Germinal epithelium, atrophy | 6 (12%) | 5 (10%) | 3 (6%) | 6 (12%) |
| Interstitial cell, hyperplasia | 12 (24%) | 18 (36%) | 19 (38%) | 9 (18%) |
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Lymph node | (7) | (10) | (8) | (14) |
| Deep cervical, ectasia | | 1 (10%) | | 1 (7%) |
| Deep cervical, hemorrhage | | | | 1 (7%) |
| Deep cervical, hyperplasia, lymphoid | | | 1 (13%) | 2 (14%) |
| Pancreatic, ectasia | | | 1 (13%) | 1 (7%) |
| Pancreatic, hyperplasia, lymphoid | | | 1 (13%) | |
| Pancreatic, inflammation, granulomatous | | | 1 (13%) | |
| Lymph node, bronchial | (7) | (12) | (11) | (8) |
| Ectasia | 1 (14%) | 1 (8%) | | |
| Hyperplasia, lymphoid | 1 (14%) | 1 (8%) | 1 (9%) | |
| Lymph node, mandibular | (1) | (3) | | (1) |
| Ectasia | 1 (100%) | 2 (67%) | | |
| Metaplasia, osseous | 1 (100%) | | | |
| Lymph node, mediastinal | (34) | (32) | (34) | (34) |
| Angiectasis | | | | 1 (3%) |
| Hemorrhage | | 1 (3%) | | |
| Hyperplasia, lymphoid | 1 (3%) | | 2 (6%) | |
| Inflammation, suppurative | 1 (3%) | | | |
| Lymph node, mesenteric | (50) | (50) | (50) | (50) |
| Hemorrhage | | 2 (4%) | | 1 (2%) |
| Spleen | (50) | (50) | (50) | (50) |
| Accessory spleen | | | | 1 (2%) |
| Fibrosis | 2 (4%) | 2 (4%) | 7 (14%) | 2 (4%) |
| Hematopoietic cell proliferation | | | | 1 (2%) |
| Hemorrhage | 4 (8%) | 4 (8%) | 2 (4%) | 4 (8%) |
| Necrosis | 1 (2%) | 3 (6%) | 1 (2%) | |
| Thymus | (49) | (49) | (50) | (50) |
| **Integumentary System** | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Galactocele | 2 (4%) | | 2 (4%) | 1 (2%) |
| Epithelium, hyperplasia | 1 (2%) | | 1 (2%) | 1 (2%) |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Integumentary System** (continued) | | | | |
| Skin | (50) | (50) | (50) | (50) |
| Cyst epithelial inclusion | 1 (2%) | 1 (2%) | | 2 (4%) |
| Hyperkeratosis | 1 (2%) | | 1 (2%) | |
| Inflammation, chronic | | | | 1 (2%) |
| Ulcer | | | | 1 (2%) |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Hyperostosis | | | 1 (2%) | |
| Skeletal muscle | (1) | (2) | (2) | (1) |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Compression | 7 (14%) | 16 (32%) | 6 (12%) | 6 (12%) |
| Hemorrhage | 5 (10%) | 7 (14%) | 3 (6%) | 1 (2%) |
| Necrosis | | 1 (2%) | | |
| Thrombosis | | 1 (2%) | | |
| **Respiratory System** | | | | |
| Larynx | (50) | (50) | (50) | (50) |
| Foreign body | 6 (12%) | 3 (6%) | | 3 (6%) |
| Inflammation, suppurative | 4 (8%) | 7 (14%) | 1 (2%) | 3 (6%) |
| Inflammation, chronic | 1 (2%) | 1 (2%) | 1 (2%) | |
| Epiglottis, hyperplasia | 1 (2%) | | | |
| Epiglottis, metaplasia, squamous | 1 (2%) | | 1 (2%) | 2 (4%) |
| Respiratory epithelium, hyperplasia | 3 (6%) | 2 (4%) | | 1 (2%) |
| Lung | (50) | (50) | (50) | (50) |
| Congestion | 1 (2%) | | | |
| Hemorrhage | 3 (6%) | 7 (14%) | 3 (6%) | |
| Inflammation | | | 1 (2%) | |
| Inflammation, suppurative | 1 (2%) | 1 (2%) | | |
| Inflammation, granulomatous | | 1 (2%) | | |
| Inflammation, chronic | 3 (6%) | 3 (6%) | 6 (12%) | 5 (10%) |
| Thrombosis | | 1 (2%) | | |
| Alveolar epithelium, hyperplasia | 13 (26%) | 11 (22%) | 10 (20%) | 10 (20%) |
| Alveolar epithelium, metaplasia, squamous | | | | 1 (2%) |
| Alveolar epithelium, metaplasia, mucous | | | | 1 (2%) |
| Alveolus, emphysema | | | | 1 (2%) |
| Alveolus, foreign body | | 1 (2%) | | |
| Alveolus, infiltration cellular, histiocyte | 9 (18%) | 7 (14%) | 4 (8%) | 14 (28%) |
| Alveolus, proteinosis | | | | 1 (2%) |
| Artery, mineralization | 2 (4%) | | | |
| Artery, thrombosis | 1 (2%) | | | |
| Bronchiole, hyperplasia | | 1 (2%) | | |
| Bronchiole, inflammation, chronic | | 1 (2%) | 1 (2%) | |
| Interstitium, fibrosis | | 2 (4%) | 1 (2%) | 1 (2%) |
| Nose | (50) | (50) | (49) | (50) |
| Foreign body | 5 (10%) | 5 (10%) | 3 (6%) | 4 (8%) |
| Hyperplasia, basal cell | | | | 1 (2%) |
| Inflammation, suppurative | 7 (14%) | 8 (16%) | 8 (16%) | 6 (12%) |
| Inflammation, chronic | 5 (10%) | 1 (2%) | | |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Respiratory System** (continued) | | | | |
| Nose (continued) | (50) | (50) | (49) | (50) |
| Epithelium, nasolacrimal duct, metaplasia, squamous | | 2 (4%) | 1 (2%) | |
| Glands, olfactory epithelium, hyperplasia | 1 (2%) | | 1 (2%) | 1 (2%) |
| Glands, respiratory epithelium, dilatation | 1 (2%) | 3 (6%) | 2 (4%) | 2 (4%) |
| Glands, respiratory epithelium, hyperplasia | | | 2 (4%) | 4 (8%) |
| Goblet cell, olfactory epithelium, hyperplasia | | | 1 (2%) | |
| Goblet cell, hyperplasia | 3 (6%) | 11 (22%) | 7 (14%) | 5 (10%) |
| Nasolacrimal duct, inflammation, suppurative | 2 (4%) | 2 (4%) | 3 (6%) | |
| Olfactory epithelium, degeneration | | | 1 (2%) | |
| Olfactory epithelium, degeneration, hyaline | 3 (6%) | 2 (4%) | 1 (2%) | |
| Olfactory epithelium, hyperplasia, basal cell | | 19 (38%) | 27 (55%) | 26 (52%) |
| Olfactory epithelium, inflammation, chronic | | | | 1 (2%) |
| Olfactory epithelium, metaplasia | 7 (14%) | 4 (8%) | 5 (10%) | 5 (10%) |
| Olfactory epithelium, necrosis | | 1 (2%) | | 1 (2%) |
| Olfactory epithelium, ulcer | | | 2 (4%) | |
| Respiratory epithelium, degeneration, hyaline | 1 (2%) | 1 (2%) | | |
| Respiratory epithelium, hyperplasia | | 15 (30%) | 16 (33%) | 23 (46%) |
| Respiratory epithelium, inflammation, chronic | | | 1 (2%) | 2 (4%) |
| Respiratory epithelium, necrosis | | | | 1 (2%) |
| Squamous epithelium, hyperplasia | | | 1 (2%) | |
| Squamous epithelium, inflammation | | | 1 (2%) | |
| Vomeronasal organ, inflammation, suppurative | 1 (2%) | | | |
| Pleura | (5) | (3) | (5) | (6) |
| Inflammation, chronic | 4 (80%) | 3 (100%) | 4 (80%) | 6 (100%) |
| Mesothelium, hyperplasia | 1 (20%) | | | |
| Trachea | (50) | (50) | (50) | (50) |
| Epithelium, hyperplasia | | | | 1 (2%) |
| Glands, cyst | | 1 (2%) | | |
| **Special Senses System** | | | | |
| Eye | (50) | (50) | (49) | (50) |
| Degeneration | | 1 (2%) | | |
| Inflammation, chronic active | | 1 (2%) | | |
| Bilateral, lens, cataract | 1 (2%) | 1 (2%) | | 1 (2%) |
| Bilateral, retina, atrophy | | 1 (2%) | 1 (2%) | |
| Ciliary body, iris, inflammation, suppurative | | | 1 (2%) | |
| Cornea, inflammation, suppurative | 2 (4%) | | | |
| Cornea, inflammation, chronic | | | | 1 (2%) |
| Lens, cataract | 5 (10%) | 1 (2%) | 2 (4%) | 4 (8%) |
| Retina, atrophy | 4 (8%) | | | 2 (4%) |
| Harderian gland | (50) | (50) | (50) | (50) |
| Inflammation, suppurative | | 1 (2%) | | |
| Inflammation, chronic | 1 (2%) | 1 (2%) | | |
| Zymbal's gland | (1) | | | (2) |
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Atrophy | | | | 1 (2%) |
| Infarct | | 1 (2%) | 2 (4%) | 1 (2%) |
| Infarct, multiple | | 1 (2%) | | |
| Nephropathy | 47 (94%) | 47 (94%) | 47 (94%) | 50 (100%) |
| Bilateral, renal tubule, cyst | | | 1 (2%) | |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Urinary System** (continued) | | | | |
| Kidney (continued) | (50) | (50) | (50) | (50) |
| Bilateral, infarct | | 1 (2%) | | |
| Capsule, dilatation | | | 1 (2%) | |
| Glomerulus, inflammation, suppurative | | 1 (2%) | | |
| Papilla, mineralization | 5 (10%) | 35 (70%) | 44 (88%) | 41 (82%) |
| Pelvis, transitional epithelium, hyperplasia | 3 (6%) | 5 (10%) | 14 (28%) | 15 (30%) |
| Pelvis, dilatation | | | 1 (2%) | 1 (2%) |
| Renal tubule, accumulation, hyaline droplet | | | 1 (2%) | 1 (2%) |
| Renal tubule, cyst | | 1 (2%) | 3 (6%) | 2 (4%) |
| Renal tubule, hyperplasia | | 3 (6%) | 8 (16%) | 6 (12%) |
| Renal tubule, hypertrophy | | | | 1 (2%) |
| Renal tubule, mineralization | 1 (2%) | | | |
| Ureter | | (1) | | (1) |
| Urethra | | | (1) | (1) |
| Transitional epithelium, hyperplasia | | | 1 (100%) | |
| Urinary bladder | (50) | (50) | (49) | (50) |
| Calculus gross observation | | | 1 (2%) | |
| Hemorrhage | | 1 (2%) | | |
| Inflammation, chronic | | | 1 (2%) | |
| Transitional epithelium, hyperplasia | 1 (2%) | | 4 (8%) | 2 (4%) |

# APPENDIX B
# SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF CUMENE

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 23 | 18 | 17 | 15 |
| Natural deaths | 6 | 5 | 2 | 3 |
| Survivors | | | | |
| Terminal sacrifice | 21 | 27 | 31 | 32 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Intestine large, cecum | (46) | (45) | (50) | (49) |
| Intestine large, colon | (48) | (46) | (50) | (50) |
| Leiomyosarcoma | | | 1 (2%) | |
| Intestine large, rectum | (48) | (46) | (50) | (50) |
| Adenoma | | | | 1 (2%) |
| Sarcoma stromal, metastatic, uterus | 1 (2%) | | | |
| Liver | (50) | (50) | (50) | (50) |
| Cholangiocarcinoma | | | | 1 (2%) |
| Fibrous histiocytoma, metastatic, skin | 1 (2%) | | | |
| Mesentery | (18) | (18) | (17) | (9) |
| Rhabdomyosarcoma | | | | 1 (11%) |
| Oral mucosa | | | (1) | (1) |
| Squamous cell carcinoma | | | | 1 (100%) |
| Pharyngeal, squamous cell carcinoma | | | 1 (100%) | |
| Pancreas | (50) | (49) | (50) | (50) |
| Rhabdomyosarcoma | | | | 1 (2%) |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Squamous cell papilloma | | | 1 (2%) | 1 (2%) |
| Stomach, glandular | (50) | (49) | (50) | (50) |
| Tongue | (1) | (2) | (2) | (3) |
| Tooth | | (2) | | |
| **Cardiovascular System** | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Osteosarcoma, metastatic, bone | | | 1 (2%) | |
| Pericardium, alveolar/bronchiolar, carcinoma, metastatic, lung | | | 1 (2%) | |
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (49) | (50) | (50) |
| Adenoma | | | | 1 (2%) |
| Osteosarcoma, metastatic, bone | | | 1 (2%) | |
| Adrenal medulla | (50) | (49) | (50) | (50) |
| Pheochromocytoma benign | 1 (2%) | | 1 (2%) | |
| Pheochromocytoma complex | 1 (2%) | | | |
| Pheochromocytoma malignant | 1 (2%) | 1 (2%) | 1 (2%) | |
| Islets, pancreatic | (50) | (49) | (50) | (50) |
| Adenoma | 1 (2%) | | | 1 (2%) |
| Carcinoma | 1 (2%) | | 1 (2%) | |
| Parathyroid gland | (49) | (45) | (48) | (47) |
| Pituitary gland | (50) | (50) | (50) | (49) |
| Adenoma | 35 (70%) | 33 (66%) | 28 (56%) | 25 (51%) |
| Carcinoma | 1 (2%) | 1 (2%) | | 1 (2%) |

**TABLE B1**
**Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Endocrine System** (continued) | | | | |
| Thyroid gland | (50) | (49) | (50) | (50) |
| Fibrous histiocytoma, metastatic, skin | 1 (2%) | | | |
| C-cell, adenoma | 3 (6%) | 4 (8%) | 2 (4%) | 2 (4%) |
| C-cell, carcinoma | | | 1 (2%) | 2 (4%) |
| Follicular cell, adenoma | | 2 (4%) | | 2 (4%) |
| Follicular cell, carcinoma | | | 1 (2%) | |
| **General Body System** | | | | |
| Peritoneum | | | (1) | |
| Carcinoma, metastatic, ovary | | | 1 (100%) | |
| **Genital System** | | | | |
| Clitoral gland | (50) | (49) | (50) | (50) |
| Adenoma | 1 (2%) | | | |
| Carcinoma | 1 (2%) | 2 (4%) | | 1 (2%) |
| Carcinoma, multiple | 1 (2%) | | | |
| Ovary | (50) | (49) | (50) | (50) |
| Cystadenocarcinoma | | | 1 (2%) | |
| Granulosa cell tumor benign | | 1 (2%) | | |
| Leiomyosarcoma, metastatic, uterus | | | | 1 (2%) |
| Osteosarcoma, metastatic, bone | | | 1 (2%) | |
| Uterus | (50) | (49) | (50) | (50) |
| Carcinoma | | 1 (2%) | | |
| Leiomyosarcoma | | | | 1 (2%) |
| Polyp stromal | 13 (26%) | 7 (14%) | 8 (16%) | 7 (14%) |
| Polyp stromal, multiple | | 1 (2%) | | |
| Sarcoma stromal | 1 (2%) | | 1 (2%) | 1 (2%) |
| Cervix, polyp stromal | | 1 (2%) | | |
| Vagina | (2) | | | |
| Polyp | 1 (50%) | | | |
| Epithelium, polyp | 1 (50%) | | | |
| **Hematopoietic System** | | | | |
| Lymph node | (3) | (3) | (5) | (2) |
| Deep cervical, squamous cell carcinoma, metastatic, skin | | | 1 (20%) | |
| Lymph node, bronchial | (7) | (9) | (8) | (5) |
| Lymph node, mandibular | (4) | (1) | (1) | (4) |
| Carcinoma, metastatic, Zymbal's gland | | | | 1 (25%) |
| Lymph node, mediastinal | (30) | (28) | (26) | (31) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (4%) | |
| Carcinoma, metastatic, thyroid gland | | | 1 (4%) | 2 (6%) |
| Squamous cell carcinoma, metastatic, skin | | | 1 (4%) | |
| Lymph node, mesenteric | (48) | (49) | (49) | (50) |
| Carcinoma, metastatic, ovary | | | 1 (2%) | |
| Spleen | (50) | (50) | (50) | (50) |
| Fibrous histiocytoma, metastatic, skin | 1 (2%) | | | |
| Rhabdomyosarcoma | | | | 1 (2%) |
| Thymus | (48) | (46) | (50) | (50) |
| Thymoma benign | | | | 1 (2%) |

**TABLE B1**
**Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Integumentary System** | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Adenoma | | 1 (2%) | | 1 (2%) |
| Carcinoma | 8 (16%) | 8 (16%) | 1 (2%) | 5 (10%) |
| Carcinoma, multiple | 2 (4%) | 1 (2%) | 1 (2%) | |
| Fibroadenoma | 17 (34%) | 11 (22%) | 24 (48%) | 21 (42%) |
| Fibroadenoma, multiple | 7 (14%) | 11 (22%) | 8 (16%) | 8 (16%) |
| Skin | (50) | (50) | (50) | (50) |
| Basal cell carcinoma | | | 1 (2%) | |
| Fibrous histiocytoma | 1 (2%) | | | |
| Keratoacanthoma | | | 2 (4%) | 1 (2%) |
| Squamous cell carcinoma | | | 1 (2%) | |
| Squamous cell papilloma | | | | 1 (2%) |
| Subcutaneous tissue, fibroma | | 2 (4%) | | 1 (2%) |
| Subcutaneous tissue, hemangiopericytoma | | | 1 (2%) | |
| Subcutaneous tissue, schwannoma benign | | 1 (2%) | | |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Cranium, osteosarcoma | | 1 (2%) | | |
| Vertebra, osteosarcoma | 1 (2%) | | 1 (2%) | |
| Skeletal muscle | (1) | | (1) | (1) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (100%) | |
| Fibrous histiocytoma, metastatic, skin | 1 (100%) | | | |
| Rhabdomyosarcoma | | | | 1 (100%) |
| **Nervous System** | | | | |
| Brain | (50) | (49) | (50) | (50) |
| Astrocytoma malignant | | | | 1 (2%) |
| Carcinoma, metastatic, pituitary gland | 1 (2%) | 2 (4%) | | 1 (2%) |
| Glioma malignant | 1 (2%) | | | |
| Granular cell tumor benign | | | 1 (2%) | |
| Meningioma benign | | 1 (2%) | | |
| Oligodendroglioma malignant | 1 (2%) | | | |
| Spinal cord | | (1) | (1) | (1) |
| **Respiratory System** | | | | |
| Larynx | (50) | (49) | (50) | (50) |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | | | | 1 (2%) |
| Alveolar/bronchiolar carcinoma | 1 (2%) | | 1 (2%) | |
| Carcinoma, metastatic, ovary | | | 1 (2%) | |
| Carcinoma, metastatic, uterus | | 1 (2%) | | |
| Fibrous histiocytoma, metastatic, skin | 1 (2%) | | | |
| Osteosarcoma, metastatic, bone | 1 (2%) | | 1 (2%) | |
| Squamous cell carcinoma, metastatic, skin | | | 1 (2%) | |
| Mediastinum, carcinoma, metastatic, uterus | | 1 (2%) | | |
| Nose | (50) | (48) | (50) | (50) |
| Nasopharyngeal duct, carcinoma, metastatic, oral mucosa | | | | 1 (2%) |
| Respiratory epithelium, adenoma | | 5 (10%) | 4 (8%) | 3 (6%) |
| Turbinate, chondroma | 1 (2%) | | | |

**TABLE B1**
**Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Respiratory System** (continued) | | | | |
| Pleura | (15) | (16) | (16) | (20) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (6%) | |
| Carcinoma, metastatic, ovary | | | 1 (6%) | |
| Trachea | (50) | (49) | (50) | (50) |
| **Special Senses System** | | | | |
| Eye | (50) | (49) | (50) | (50) |
| Harderian gland | (50) | (49) | (50) | (50) |
| Zymbal's gland | (1) | | (1) | (1) |
| Carcinoma | 1 (100%) | | 1 (100%) | 1 (100%) |
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Urinary bladder | (50) | (49) | (50) | (50) |
| Leiomyosarcoma | 1 (2%) | | | |
| **Systemic Lesions** | | | | |
| Multiple organs[b] | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | | 1 (2%) | | |
| Leukemia mononuclear | 12 (24%) | 25 (50%) | 23 (46%) | 13 (26%) |
| Lymphoma malignant | | | | 1 (2%) |
| **Neoplasm Summary** | | | | |
| Total animals with primary neoplasms[c] | 50 | 48 | 48 | 47 |
| Total primary neoplasms | 117 | 122 | 118 | 111 |
| Total animals with benign neoplasms | 44 | 42 | 43 | 43 |
| Total benign neoplasms | 81 | 81 | 80 | 78 |
| Total animals with malignant neoplasms | 24 | 32 | 30 | 25 |
| Total malignant neoplasms | 36 | 41 | 38 | 33 |
| Total animals with metastatic neoplasms | 4 | 3 | 5 | 6 |
| Total metastatic neoplasms | 8 | 4 | 16 | 6 |

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

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma** | | | | |
| Overall rate[a] | 3/50 (6%) | 1/49 (2%) | 2/50 (4%) | 0/50 (0%) |
| Adjusted rate[b] | 7.3% | 2.4% | 4.7% | 0.0% |
| Terminal rate[c] | 1/21 (5%) | 1/27 (4%) | 2/31 (7%) | 0/32 (0%) |
| First incidence (days) | 716 | 730 (T) | 730 (T) | —[e] |
| Poly-3 test[d] | P=0.096N | P=0.300N | P=0.479N | P=0.109N |
| **Clitoral Gland: Adenoma or Carcinoma** | | | | |
| Overall rate | 3/50 (6%) | 2/49 (4%) | 0/50 (0%) | 1/50 (2%) |
| Adjusted rate | 7.2% | 4.8% | 0.0% | 2.3% |
| Terminal rate | 1/21 (5%) | 1/27 (4%) | 0/31 (0%) | 1/32 (3%) |
| First incidence (days) | 501 | 709 | — | 730 (T) |
| Poly-3 test | P=0.158N | P=0.502N | P=0.113N | P=0.292N |
| **Mammary Gland: Fibroadenoma** | | | | |
| Overall rate | 24/50 (48%) | 22/50 (44%) | 32/50 (64%) | 29/50 (58%) |
| Adjusted rate | 54.9% | 49.9% | 70.4% | 63.4% |
| Terminal rate | 11/21 (52%) | 15/27 (56%) | 22/31 (71%) | 19/32 (59%) |
| First incidence (days) | 501 | 571 | 530 | 619 |
| Poly-3 test | P=0.122 | P=0.396N | P=0.087 | P=0.267 |
| **Mammary Gland: Fibroadenoma or Adenoma** | | | | |
| Overall rate | 24/50 (48%) | 23/50 (46%) | 32/50 (64%) | 29/50 (58%) |
| Adjusted rate | 54.9% | 51.9% | 70.4% | 63.4% |
| Terminal rate | 11/21 (52%) | 15/27 (56%) | 22/31 (71%) | 19/32 (59%) |
| First incidence (days) | 501 | 571 | 530 | 619 |
| Poly-3 test | P=0.138 | P=0.472N | P=0.087 | P=0.267 |
| **Mammary Gland: Carcinoma** | | | | |
| Overall rate | 10/50 (20%) | 9/50 (18%) | 2/50 (4%) | 5/50 (10%) |
| Adjusted rate | 23.9% | 21.2% | 4.7% | 11.2% |
| Terminal rate | 5/21 (24%) | 8/27 (30%) | 1/31 (3%) | 1/32 (3%) |
| First incidence (days) | 558 | 704 | 715 | 542 |
| Poly-3 test | P=0.038N | P=0.484N | P=0.011N | P=0.101N |
| **Mammary Gland: Adenoma or Carcinoma** | | | | |
| Overall rate | 10/50 (20%) | 10/50 (20%) | 2/50 (4%) | 6/50 (12%) |
| Adjusted rate | 23.9% | 23.4% | 4.7% | 13.4% |
| Terminal rate | 5/21 (24%) | 8/27 (30%) | 1/31 (3%) | 1/32 (3%) |
| First incidence (days) | 558 | 673 | 715 | 542 |
| Poly-3 test | P=0.063N | P=0.580N | P=0.011N | P=0.162N |
| **Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma** | | | | |
| Overall rate | 31/50 (62%) | 29/50 (58%) | 32/50 (64%) | 33/50 (66%) |
| Adjusted rate | 69.9% | 65.3% | 70.4% | 70.5% |
| Terminal rate | 16/21 (76%) | 20/27 (74%) | 22/31 (71%) | 20/32 (63%) |
| First incidence (days) | 501 | 571 | 530 | 542 |
| Poly-3 test | P=0.444 | P=0.400N | P=0.576 | P=0.570 |
| **Nose: Adenoma** | | | | |
| Overall rate | 0/50 (0%) | 5/48 (10%) | 4/50 (8%) | 3/50 (6%) |
| Adjusted rate | 0.0% | 12.2% | 9.3% | 6.9% |
| Terminal rate | 0/21 (0%) | 5/27 (19%) | 4/31 (13%) | 2/32 (6%) |
| First incidence (days) | — | 730 (T) | 730 (T) | 638 |
| Poly-3 test | P=0.320 | P=0.030 | P=0.066 | P=0.130 |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Pituitary Gland (Pars Distalis): Adenoma** | | | | |
| Overall rate | 35/50 (70%) | 33/50 (66%) | 28/50 (56%) | 25/49 (51%) |
| Adjusted rate | 77.1% | 71.4% | 61.4% | 56.2% |
| Terminal rate | 17/21 (81%) | 17/27 (63%) | 19/31 (61%) | 17/32 (53%) |
| First incidence (days) | 501 | 536 | 528 | 542 |
| Poly-3 test | P=0.013N | P=0.342N | P=0.070N | P=0.023N |
| **Pituitary Gland (Pars Distalis): Adenoma or Carcinoma** | | | | |
| Overall rate | 36/50 (72%) | 34/50 (68%) | 28/50 (56%) | 26/49 (53%) |
| Adjusted rate | 78.9% | 72.7% | 61.4% | 58.1% |
| Terminal rate | 17/21 (81%) | 17/27 (63%) | 19/31 (61%) | 17/32 (53%) |
| First incidence (days) | 501 | 536 | 528 | 542 |
| Poly-3 test | P=0.012N | P=0.319N | P=0.045N | P=0.022N |
| **Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma** | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate | 0.0% | 0.0% | 6.8% | 4.6% |
| Terminal rate | 0/21 (0%) | 0/27 (0%) | 1/31 (3%) | 1/32 (3%) |
| First incidence (days) | — | — | 516 | 683 |
| Poly-3 test | P=0.106 | — | P=0.132 | P=0.250 |
| **Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma** | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 4/50 (8%) | 2/50 (4%) |
| Adjusted rate | 0.0% | 0.0% | 9.1% | 4.6% |
| Terminal rate | 0/21 (0%) | 0/27 (0%) | 2/31 (7%) | 1/32 (3%) |
| First incidence (days) | — | — | 516 | 683 |
| Poly-3 test | P=0.114 | —[f] | P=0.069 | P=0.250 |
| **Thyroid Gland (C-Cell): Adenoma** | | | | |
| Overall rate | 3/50 (6%) | 4/49 (8%) | 2/50 (4%) | 2/50 (4%) |
| Adjusted rate | 7.3% | 9.5% | 4.7% | 4.6% |
| Terminal rate | 2/21 (10%) | 2/27 (7%) | 2/31 (7%) | 2/32 (6%) |
| First incidence (days) | 716 | 655 | 730 (T) | 730 (T) |
| Poly-3 test | P=0.295N | P=0.513 | P=0.479N | P=0.476N |
| **Thyroid Gland (C-Cell): Adenoma or Carcinoma** | | | | |
| Overall rate | 3/50 (6%) | 4/49 (8%) | 3/50 (6%) | 4/50 (8%) |
| Adjusted rate | 7.3% | 9.5% | 7.0% | 9.3% |
| Terminal rate | 2/21 (10%) | 2/27 (7%) | 3/31 (10%) | 4/32 (13%) |
| First incidence (days) | 716 | 655 | 730 (T) | 730 (T) |
| Poly-3 test | P=0.495 | P=0.513 | P=0.641N | P=0.529 |
| **Uterus: Stromal Polyp** | | | | |
| Overall rate | 13/50 (26%) | 9/50 (18%) | 8/50 (16%) | 7/50 (14%) |
| Adjusted rate | 31.1% | 21.1% | 18.2% | 15.9% |
| Terminal rate | 10/21 (48%) | 7/27 (26%) | 6/31 (19%) | 4/32 (13%) |
| First incidence (days) | 547 | 696 | 528 | 628 |
| Poly-3 test | P=0.070N | P=0.210N | P=0.125N | P=0.076N |
| **Uterus: Stromal Polyp or Stromal Sarcoma** | | | | |
| Overall rate | 14/50 (28%) | 9/50 (18%) | 9/50 (18%) | 8/50 (16%) |
| Adjusted rate | 33.5% | 21.1% | 20.3% | 17.9% |
| Terminal rate | 10/21 (48%) | 7/27 (26%) | 6/31 (19%) | 4/32 (13%) |
| First incidence (days) | 547 | 696 | 528 | 411 |
| Poly-3 test | P=0.081N | P=0.148N | P=0.124N | P=0.075N |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **All Organs: Mononuclear Leukemia** | | | | |
| Overall rate | 12/50 (24%) | 25/50 (50%) | 23/50 (46%) | 13/50 (26%) |
| Adjusted rate | 27.9% | 55.5% | 50.2% | 29.0% |
| Terminal rate | 3/21 (14%) | 14/27 (52%) | 12/31 (39%) | 7/32 (22%) |
| First incidence (days) | 492 | 547 | 530 | 542 |
| Poly-3 test | P=0.277N | P=0.006 | P=0.023 | P=0.548 |
| **All Organs: Benign Neoplasms** | | | | |
| Overall rate | 44/50 (88%) | 42/50 (84%) | 43/50 (86%) | 43/50 (86%) |
| Adjusted rate | 93.2% | 89.8% | 89.7% | 91.8% |
| Terminal rate | 21/21 (100%) | 24/27 (89%) | 28/31 (90%) | 30/32 (94%) |
| First incidence (days) | 374 | 536 | 516 | 542 |
| Poly-3 test | P=0.519N | P=0.399N | P=0.390N | P=0.559N |
| **All Organs: Malignant Neoplasms** | | | | |
| Overall rate | 24/50 (48%) | 33/50 (66%) | 30/50 (60%) | 25/50 (50%) |
| Adjusted rate | 53.0% | 70.8% | 62.1% | 52.7% |
| Terminal rate | 7/21 (33%) | 19/27 (70%) | 15/31 (48%) | 13/32 (41%) |
| First incidence (days) | 485 | 514 | 339 | 267 |
| Poly-3 test | P=0.304N | P=0.053 | P=0.242 | P=0.573N |
| **All Organs: Benign or Malignant Neoplasms** | | | | |
| Overall rate | 50/50 (100%) | 48/50 (96%) | 48/50 (96%) | 47/50 (94%) |
| Adjusted rate | 100% | 98.0% | 96.0% | 95.6% |
| Terminal rate | 21/21 (100%) | 26/27 (96%) | 29/31 (94%) | 30/32 (94%) |
| First incidence (days) | 374 | 514 | 339 | 267 |
| Poly-3 test | P=0.132N | P=0.496N | P=0.237N | P=0.200N |

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, nose, 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 are the P values 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 B3**
**Historical Incidence of Adenoma of the Nose in Control Female F344/N Rats[a]**

| Study | Incidence in Controls |
|---|---|
| **Historical Incidence: Inhalation Studies** | |
| Cumene | 0/50 |
| Decalin | 0/50 |
| Divinylbenzene | 0/50 |
| Methyl isobutyl ketone | 0/50 |
| α-Methylstyrene | 0/49 |
| Propargyl alcohol | 0/49 |
| Propylene glycol mono-*t*-butyl ether | 0/49 |
| Stoddard solvent IIC | 0/49 |
| Vanadium pentoxide | 0/50 |
| **Overall Historical Incidence: Inhalation Studies** | |
| Total | 0/496 |
| **Overall Historical Incidence: All Routes** | |
| Total (%) | 0/1,343 |

[a] Data as of March 2, 2007

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 23 | 18 | 17 | 15 |
| Natural deaths | 6 | 5 | 2 | 3 |
| Survivors | | | | |
| Terminal sacrifice | 21 | 27 | 31 | 32 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Intestine large, cecum | (46) | (45) | (50) | (49) |
| Necrosis | | | | 1 (2%) |
| Intestine large, colon | (48) | (46) | (50) | (50) |
| Artery, inflammation, chronic | | | | 1 (2%) |
| Intestine large, rectum | (48) | (46) | (50) | (50) |
| Liver | (50) | (50) | (50) | (50) |
| Angiectasis | 1 (2%) | | 2 (4%) | |
| Basophilic focus | 27 (54%) | 31 (62%) | 36 (72%) | 32 (64%) |
| Clear cell focus | 15 (30%) | 9 (18%) | 6 (12%) | 5 (10%) |
| Eosinophilic focus | | | | 1 (2%) |
| Hepatodiaphragmatic nodule | 6 (12%) | 6 (12%) | 4 (8%) | 6 (12%) |
| Inflammation, granulomatous | 1 (2%) | | | |
| Mixed cell focus | | | 2 (4%) | 1 (2%) |
| Necrosis | | 1 (2%) | 1 (2%) | 3 (6%) |
| Vacuolization cytoplasmic | 11 (22%) | 4 (8%) | 4 (8%) | 2 (4%) |
| Bile duct, hyperplasia | | | 1 (2%) | |
| Centrilobular, congestion | | 1 (2%) | | |
| Periportal, vacuolization cytoplasmic | 1 (2%) | | | |
| Mesentery | (18) | (18) | (17) | (9) |
| Necrosis | 18 (100%) | 16 (89%) | 17 (100%) | 8 (89%) |
| Oral mucosa | | | (1) | (1) |
| Pancreas | (50) | (49) | (50) | (50) |
| Fibrosis | | 1 (2%) | | |
| Hemorrhage | | 1 (2%) | | |
| Thrombosis | | | | 1 (2%) |
| Artery, inflammation, chronic | | | | 1 (2%) |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Erosion | | | 1 (2%) | |
| Inflammation, suppurative | | | | 1 (2%) |
| Inflammation, chronic | | 1 (2%) | 1 (2%) | |
| Ulcer | 5 (10%) | 4 (8%) | 3 (6%) | 1 (2%) |
| Epithelium, cyst | | | 1 (2%) | |
| Epithelium, erosion | | 1 (2%) | | |
| Epithelium, hyperplasia | 11 (22%) | 13 (26%) | 12 (24%) | 8 (16%) |
| Submucosa, fibrosis | | | 1 (2%) | |
| Stomach, glandular | (50) | (49) | (50) | (50) |
| Erosion | 1 (2%) | | | |
| Ulcer | | | 1 (2%) | |
| Epithelium, hyperplasia | | | | 1 (2%) |
| Tongue | (1) | (2) | (2) | (3) |
| Epithelium, hyperplasia | 1 (100%) | 2 (100%) | 2 (100%) | 3 (100%) |
| Tooth | | (2) | | |
| Peridontal tissue, inflammation | | 2 (100%) | | |

[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 Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Cardiovascular System** | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Cardiomyopathy | 1 (2%) | 2 (4%) | 2 (4%) | |
| Inflammation, chronic | | | | 1 (2%) |
| Atrium, thrombosis | | 2 (4%) | 2 (4%) | 1 (2%) |
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (49) | (50) | (50) |
| Hemorrhage | 1 (2%) | | | |
| Hyperplasia | 9 (18%) | 9 (18%) | 13 (26%) | 12 (24%) |
| Necrosis | 2 (4%) | | | 1 (2%) |
| Vacuolization cytoplasmic | 11 (22%) | 18 (37%) | 16 (32%) | 7 (14%) |
| Adrenal medulla | (50) | (49) | (50) | (50) |
| Islets, pancreatic | (50) | (49) | (50) | (50) |
| Parathyroid gland | (49) | (45) | (48) | (47) |
| Hyperplasia | | 1 (2%) | | |
| Pituitary gland | (50) | (50) | (50) | (49) |
| Cyst | 7 (14%) | 2 (4%) | 2 (4%) | 2 (4%) |
| Hemorrhage | 2 (4%) | 2 (4%) | 2 (4%) | |
| Hyperplasia | 10 (20%) | 13 (26%) | 6 (12%) | 16 (33%) |
| Thyroid gland | (50) | (49) | (50) | (50) |
| Ultimobranchial cyst | 1 (2%) | | | |
| C-cell, hyperplasia | 13 (26%) | 13 (27%) | 15 (30%) | 15 (30%) |
| Follicular cell, hyperplasia | | | 1 (2%) | |
| **General Body System** | | | | |
| Peritoneum | | | (1) | |
| **Genital System** | | | | |
| Clitoral gland | (50) | (49) | (50) | (50) |
| Cyst | | 2 (4%) | 2 (4%) | 1 (2%) |
| Hyperplasia | 2 (4%) | 5 (10%) | 7 (14%) | 5 (10%) |
| Inflammation, chronic | | 1 (2%) | 1 (2%) | 3 (6%) |
| Ovary | (50) | (49) | (50) | (50) |
| Cyst | 3 (6%) | 7 (14%) | 7 (14%) | 6 (12%) |
| Cyst, multiple | | | 1 (2%) | |
| Uterus | (50) | (49) | (50) | (50) |
| Hemorrhage | 1 (2%) | 1 (2%) | 4 (8%) | 1 (2%) |
| Pigmentation | | | 1 (2%) | |
| Endometrium, hyperplasia | 4 (8%) | 7 (14%) | 2 (4%) | 5 (10%) |
| Vagina | (2) | | | |
| Epithelium, hyperplasia, adenomatous | 1 (50%) | | | |
| **Hematopoietic System** | | | | |
| Lymph node | (3) | (3) | (5) | (2) |
| Pancreatic, infiltration cellular, histiocyte | | | 1 (20%) | |
| Pancreatic, pigmentation | | | 1 (20%) | |
| Lymph node, bronchial | (7) | (9) | (8) | (5) |
| Ectasia | 2 (29%) | | | |
| Hemorrhage | | | | 1 (20%) |
| Hyperplasia, lymphoid | | 1 (11%) | | 1 (20%) |
| Inflammation | 1 (14%) | | | |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Hematopoietic System** (continued) | | | | |
| Lymph node, mandibular | (4) | (1) | (1) | (4) |
| Ectasia | | | | 1 (25%) |
| Hyperplasia, lymphoid | | | | 1 (25%) |
| Lymph node, mediastinal | (30) | (28) | (26) | (31) |
| Angiectasis | | | 1 (4%) | |
| Hemorrhage | | | 1 (4%) | 1 (3%) |
| Hyperplasia, histiocytic | | | 1 (4%) | |
| Hyperplasia, lymphoid | | | 1 (4%) | 2 (6%) |
| Inflammation, suppurative | | | | 1 (3%) |
| Lymph node, mesenteric | (48) | (49) | (49) | (50) |
| Ectasia | | | 1 (2%) | |
| Hemorrhage | 2 (4%) | | | |
| Hyperplasia, lymphoid | | 1 (2%) | | |
| Infiltration cellular, histiocyte | | 1 (2%) | | |
| Necrosis | | | | 1 (2%) |
| Spleen | (50) | (50) | (50) | (50) |
| Atrophy | | | | 1 (2%) |
| Fibrosis | | | 3 (6%) | |
| Hematopoietic cell proliferation | | 1 (2%) | 2 (4%) | 2 (4%) |
| Necrosis | | | | 2 (4%) |
| Capsule, fibrosis | 1 (2%) | | | |
| Thymus | (48) | (46) | (50) | (50) |
| Hyperplasia, tubular | | | 1 (2%) | |
| **Integumentary System** | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Galactocele | 3 (6%) | | | 2 (4%) |
| Hyperplasia | 1 (2%) | | | |
| Inflammation, suppurative | | 1 (2%) | | |
| Epithelium, hyperplasia | | | 1 (2%) | 1 (2%) |
| Skin | (50) | (50) | (50) | (50) |
| Cyst epithelial inclusion | | 1 (2%) | | |
| Hyperkeratosis | 1 (2%) | 1 (2%) | | 1 (2%) |
| Inflammation, chronic | | | | 1 (2%) |
| Ulcer | | 3 (6%) | | 2 (4%) |
| Sebaceous gland, hemorrhage | | 1 (2%) | | |
| Subcutaneous tissue, fibrosis | | | | 1 (2%) |
| Subcutaneous tissue, inflammation, suppurative | | 1 (2%) | | |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Hyperostosis | | 1 (2%) | | |
| Maxilla, fracture | | | 1 (2%) | |
| Maxilla, inflammation, chronic active | | | | 1 (2%) |
| Skeletal muscle | (1) | | (1) | (1) |
| **Nervous System** | | | | |
| Brain | (50) | (49) | (50) | (50) |
| Compression | 12 (24%) | 9 (18%) | 4 (8%) | 5 (10%) |
| Demyelination | | | | 1 (2%) |
| Gliosis | | | | 1 (2%) |
| Hemorrhage | 1 (2%) | 3 (6%) | 2 (4%) | 3 (6%) |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Nervous System** (continued) | | | | |
| Brain (continued) | (50) | (49) | (50) | (50) |
| Inflammation, chronic active | | | | 1 (2%) |
| Necrosis | 1 (2%) | | | |
| Thrombosis | | 1 (2%) | 1 (2%) | |
| Medulla, meninges, inflammation, suppurative | 1 (2%) | | | |
| Medulla, neuron, necrosis | | | | 1 (2%) |
| Neuron, degeneration | | | | 1 (2%) |
| Spinal cord | | (1) | (1) | (1) |
| Gliosis | | | | 1 (100%) |
| Inflammation, chronic active | | | | 1 (100%) |
| **Respiratory System** | | | | |
| Larynx | (50) | (49) | (50) | (50) |
| Foreign body | 2 (4%) | 1 (2%) | 2 (4%) | |
| Inflammation, suppurative | 3 (6%) | 3 (6%) | 4 (8%) | 1 (2%) |
| Inflammation, chronic | 2 (4%) | | 2 (4%) | 2 (4%) |
| Respiratory epithelium, hyperplasia | | 1 (2%) | | 1 (2%) |
| Lung | (50) | (50) | (50) | (50) |
| Cyst, squamous | | | | 1 (2%) |
| Hemorrhage | 2 (4%) | 1 (2%) | 2 (4%) | |
| Inflammation, suppurative | 1 (2%) | | | |
| Inflammation, chronic | 11 (22%) | 10 (20%) | 8 (16%) | 16 (32%) |
| Thrombosis | | | 1 (2%) | |
| Alveolar epithelium, hyperplasia | 3 (6%) | 1 (2%) | 5 (10%) | 7 (14%) |
| Alveolar epithelium, metaplasia, squamous | | | | 1 (2%) |
| Alveolus, infiltration cellular, histiocyte | 16 (32%) | 6 (12%) | 11 (22%) | 14 (28%) |
| Alveolus, proteinosis | | | | 2 (4%) |
| Bronchiole, hyperplasia | | 3 (6%) | | 1 (2%) |
| Bronchiole, inflammation, chronic | 1 (2%) | 1 (2%) | | 3 (6%) |
| Interstitium, fibrosis | 1 (2%) | | | |
| Nose | (50) | (48) | (50) | (50) |
| Foreign body | | 4 (8%) | 2 (4%) | 3 (6%) |
| Hyperplasia, basal cell | | | | 1 (2%) |
| Inflammation, suppurative | 1 (2%) | 3 (6%) | 1 (2%) | 2 (4%) |
| Inflammation, chronic | 1 (2%) | 3 (6%) | 4 (8%) | 1 (2%) |
| Inflammation, chronic active | | | 1 (2%) | |
| Glands, respiratory epithelium, dilatation | 1 (2%) | | | |
| Goblet cell, hyperplasia | 4 (8%) | 6 (13%) | 1 (2%) | 5 (10%) |
| Nasolacrimal duct, inflammation, suppurative | 4 (8%) | 3 (6%) | 2 (4%) | 1 (2%) |
| Nasolacrimal duct, inflammation, chronic | 1 (2%) | | | |
| Olfactory epithelium, degeneration, hyaline | 2 (4%) | 1 (2%) | | |
| Olfactory epithelium, hyperplasia | | | 1 (2%) | |
| Olfactory epithelium, hyperplasia, basal cell | | 14 (29%) | 25 (50%) | 31 (62%) |
| Olfactory epithelium, inflammation, granulomatous | | 1 (2%) | | |
| Olfactory epithelium, metaplasia | | 1 (2%) | | 2 (4%) |
| Olfactory epithelium, necrosis | | | 1 (2%) | |
| Respiratory epithelium, degeneration, hyaline | 1 (2%) | | 3 (6%) | |
| Respiratory epithelium, hyperplasia | | | 4 (8%) | 6 (12%) |
| Respiratory epithelium, metaplasia, squamous | | 1 (2%) | | |
| Turbinate, necrosis | | | 1 (2%) | |
| Pleura | (15) | (16) | (16) | (20) |
| Inflammation, chronic | 15 (100%) | 15 (94%) | 14 (88%) | 20 (100%) |
| Mesothelium, hyperplasia | | 1 (6%) | | |
| Trachea | (50) | (49) | (50) | (50) |
| Glands, cyst | | | 1 (2%) | |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Special Senses System** | | | | |
| Eye | (50) | (49) | (50) | (50) |
| Bilateral, lens, cataract | 1 (2%) | | | |
| Bilateral, retina, atrophy | 1 (2%) | 1 (2%) | 1 (2%) | |
| Cornea, epithelium, hyperplasia | | | | 1 (2%) |
| Cornea, inflammation | | | | 1 (2%) |
| Cornea, inflammation, suppurative | 1 (2%) | | | |
| Lens, cataract | 5 (10%) | 2 (4%) | 5 (10%) | 3 (6%) |
| Retina, atrophy | 3 (6%) | 5 (10%) | 8 (16%) | 2 (4%) |
| Harderian gland | (50) | (49) | (50) | (50) |
| Inflammation, chronic | 1 (2%) | | 1 (2%) | 1 (2%) |
| Zymbal's gland | (1) | | (1) | (1) |
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Infarct, multiple | | | 1 (2%) | |
| Nephropathy | 38 (76%) | 37 (74%) | 41 (82%) | 44 (88%) |
| Artery, inflammation, chronic active | | | | 1 (2%) |
| Papilla, mineralization | 6 (12%) | 3 (6%) | 4 (8%) | 6 (12%) |
| Pelvis, transitional epithelium, hyperplasia | 1 (2%) | 1 (2%) | 6 (12%) | 1 (2%) |
| Pelvis, transitional epithelium, mineralization | 23 (46%) | 27 (54%) | 27 (54%) | 22 (44%) |
| Pelvis, dilatation | 1 (2%) | | | |
| Renal tubule, accumulation, hyaline droplet | 1 (2%) | | 1 (2%) | |
| Renal tubule, cyst | 1 (2%) | | 1 (2%) | 1 (2%) |
| Renal tubule, pigmentation | 1 (2%) | | | |
| Urinary bladder | (50) | (49) | (50) | (50) |
| Transitional epithelium, hyperplasia | 1 (2%) | 2 (4%) | 2 (4%) | 1 (2%) |

# APPENDIX C
# SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR INHALATION STUDY OF CUMENE

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 7 | 9 | 13 | 20 |
| Natural deaths | 5 | 7 | 7 | 7 |
| Survivors | | | | |
| Terminal sacrifice | 38 | 34 | 30 | 23 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Gallbladder | (43) | (32) | (37) | (38) |
| Intestine large, cecum | (49) | (47) | (44) | (44) |
| Mast cell tumor malignant, metastatic, uncertain primary site | | 1 (2%) | | |
| Intestine large, colon | (50) | (46) | (45) | (48) |
| Leiomyoma | | 1 (2%) | | |
| Intestine small, duodenum | (49) | (44) | (43) | (44) |
| Carcinoma | | 1 (2%) | | |
| Intestine small, ileum | (49) | (46) | (43) | (44) |
| Intestine small, jejunum | (48) | (46) | (43) | (44) |
| Carcinoma | 1 (2%) | 1 (2%) | | 2 (5%) |
| Liver | (50) | (50) | (50) | (50) |
| Hemangioma | 1 (2%) | | | |
| Hemangiosarcoma | | 1 (2%) | 1 (2%) | 1 (2%) |
| Hepatoblastoma | 1 (2%) | 1 (2%) | 4 (8%) | 3 (6%) |
| Hepatocellular adenoma | 17 (34%) | 13 (26%) | 15 (30%) | 9 (18%) |
| Hepatocellular adenoma, multiple | 17 (34%) | 20 (40%) | 22 (44%) | 26 (52%) |
| Hepatocellular carcinoma | 10 (20%) | 17 (34%) | 17 (34%) | 10 (20%) |
| Hepatocellular carcinoma, multiple | 3 (6%) | 1 (2%) | 4 (8%) | 7 (14%) |
| Hepatocholangiocarcinoma | | | | 1 (2%) |
| Sarcoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Mesentery | (3) | (7) | (6) | (3) |
| Mast cell tumor malignant, metastatic, uncertain primary site | | 1 (14%) | | |
| Pancreas | (50) | (50) | (49) | (49) |
| Sarcoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Stomach, forestomach | (50) | (50) | (50) | (49) |
| Mast cell tumor malignant, metastatic, uncertain primary site | 1 (2%) | 1 (2%) | | |
| Sarcoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Squamous cell carcinoma | | | 1 (2%) | |
| Squamous cell papilloma | 1 (2%) | | | |
| Stomach, glandular | (50) | (48) | (48) | (48) |
| Sarcoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Tongue | (1) | | | |
| Squamous cell carcinoma | 1 (100%) | | | |
| Tooth | (15) | (8) | (8) | (6) |
| Mast cell tumor malignant, metastatic, uncertain primary site | 1 (7%) | 1 (13%) | | |

**TABLE C1**
**Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Cardiovascular System** | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (2%) | 1 (2%) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Hemangiosarcoma | | | 1 (2%) | |
| Hepatocellular carcinoma, metastatic, liver | | | 1 (2%) | |
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (49) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | 1 (2%) | | |
| Sarcoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Subcapsular, adenoma | 1 (2%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Adrenal medulla | (50) | (50) | (48) | (50) |
| Pheochromocytoma benign | | 1 (2%) | 1 (2%) | |
| Pheochromocytoma malignant | | 1 (2%) | | |
| Islets, pancreatic | (50) | (50) | (49) | (49) |
| Pituitary gland | (48) | (49) | (49) | (49) |
| Pars distalis, adenoma | 1 (2%) | | | 1 (2%) |
| Thyroid gland | (50) | (50) | (49) | (50) |
| Follicular cell, adenoma | | | | 3 (6%) |
| **General Body System** | | | | |
| None | | | | |
| **Genital System** | | | | |
| Epididymis | (50) | (50) | (49) | (50) |
| Preputial gland | (49) | (50) | (50) | (50) |
| Prostate | (50) | (49) | (48) | (49) |
| Testes | (50) | (50) | (49) | (50) |
| Interstitial cell, adenoma | 1 (2%) | | | |
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (49) | (49) |
| Lymph node | | | (1) | (2) |
| Pancreatic, sarcoma, metastatic, uncertain primary site | | | | 1 (50%) |
| Renal, carcinoma, metastatic, uncertain primary site | | | | 1 (50%) |
| Lymph node, bronchial | (45) | (41) | (45) | (35) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (3%) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (3%) |
| Sarcoma, metastatic, uncertain primary site | | | | 1 (3%) |
| Lymph node, mandibular | (34) | (35) | (22) | (25) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (4%) |
| Mast cell tumor metastatic, uncertain primary site | 1 (3%) | | | |
| Lymph node, mediastinal | (37) | (42) | (40) | (39) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (3%) | 2 (5%) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (3%) |
| Hepatocellular carcinoma, metastatic, liver | | | | 1 (3%) |
| Sarcoma, metastatic, uncertain primary site | | | | 1 (3%) |
| Lymph node, mesenteric | (48) | (47) | (46) | (44) |
| Sarcoma, metastatic, uncertain primary site | | | | 1 (2%) |

**TABLE C1**
**Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Hematopoietic System** (continued) | | | | |
| Spleen | (50) | (50) | (49) | (50) |
| Hemangiosarcoma | | | | 4 (8%) |
| Sarcoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Thymus | (45) | (44) | (38) | (44) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (2%) |
| Mast cell tumor malignant, metastatic, uncertain primary site | 1 (2%) | | | |
| Sarcoma, metastatic, uncertain primary site | | | | 1 (2%) |
| **Integumentary System** | | | | |
| Skin | (50) | (50) | (49) | (50) |
| Hepatocellular carcinoma, metastatic, liver | | 1 (2%) | | |
| Subcutaneous tissue, fibrosarcoma | | 1 (2%) | | |
| Subcutaneous tissue, fibrous histiocytoma | 1 (2%) | 2 (4%) | 1 (2%) | 3 (6%) |
| Subcutaneous tissue, sarcoma | 1 (2%) | | | |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Skeletal muscle | | | (1) | |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (100%) | |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (2%) |
| **Respiratory System** | | | | |
| Larynx | (50) | (49) | (50) | (50) |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | 12 (24%) | 19 (38%) | 16 (32%) | 9 (18%) |
| Alveolar/bronchiolar adenoma, multiple | 1 (2%) | 12 (24%) | 15 (30%) | 20 (40%) |
| Alveolar/bronchiolar carcinoma | 9 (18%) | 11 (22%) | 12 (24%) | 16 (32%) |
| Alveolar/bronchiolar carcinoma, multiple | | 8 (16%) | 20 (40%) | 17 (34%) |
| Carcinoma, metastatic, Harderian gland | 1 (2%) | | 2 (4%) | 1 (2%) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Hepatocellular carcinoma, metastatic, liver | 7 (14%) | 8 (16%) | 11 (22%) | 7 (14%) |
| Sarcoma, metastatic, uncertain primary site | | | 1 (2%) | 1 (2%) |
| Nose | (50) | (50) | (49) | (48) |
| Pleura | | | | (2) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (50%) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (50%) |
| Trachea | (50) | (50) | (49) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (2%) |
| **Special Senses System** | | | | |
| Eye | (49) | (50) | (49) | (48) |
| Harderian gland | (48) | (50) | (49) | (50) |
| Adenoma | 8 (17%) | 2 (4%) | 3 (6%) | 4 (8%) |
| Carcinoma | 4 (8%) | 3 (6%) | 2 (4%) | 2 (4%) |
| Bilateral, adenoma | | | 1 (2%) | 1 (2%) |

**TABLE C1**
**Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (2%) | 1 (2%) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Hepatocellular carcinoma, metastatic, liver | 1 (2%) | 1 (2%) | | |
| Mast cell tumor malignant, metastatic, uncertain primary site | 1 (2%) | 1 (2%) | | |
| Capsule, sarcoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Urinary bladder | (50) | (48) | (49) | (48) |
| Hemangiosarcoma | | | 1 (2%) | |
| **Systemic Lesions** | | | | |
| Multiple organs[b] | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | | 1 (2%) | | |
| Lymphoma malignant | 2 (4%) | 1 (2%) | 1 (2%) | |
| **Neoplasm Summary** | | | | |
| Total animals with primary neoplasms[c] | 48 | 50 | 50 | 50 |
| Total primary neoplasms | 93 | 119 | 140 | 140 |
| Total animals with benign neoplasms | 43 | 45 | 46 | 44 |
| Total benign neoplasms | 60 | 69 | 75 | 74 |
| Total animals with malignant neoplasms | 26 | 34 | 47 | 45 |
| Total malignant neoplasms | 33 | 50 | 65 | 66 |
| Total animals with metastatic neoplasms | 9 | 10 | 15 | 12 |
| Total metastatic neoplasms | 14 | 16 | 19 | 40 |
| Total animals with malignant neoplasms-uncertain primary site | 1 | 1 | 1 | 2 |

[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 Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Harderian Gland: Adenoma** | | | | |
| Overall rate[a] | 8/50 (16%) | 2/50 (4%) | 4/50 (8%) | 5/50 (10%) |
| Adjusted rate[b] | 17.2% | 4.5% | 9.0% | 12.2% |
| Terminal rate[c] | 8/38 (21%) | 2/34 (6%) | 3/30 (10%) | 2/23 (9%) |
| First incidence (days) | 729 (T) | 729 (T) | 565 | 556 |
| Poly-3 test[d] | P=0.400N | P=0.052N | P=0.195N | P=0.358N |
| **Harderian Gland: Carcinoma** | | | | |
| Overall rate | 4/50 (8%) | 3/50 (6%) | 2/50 (4%) | 2/50 (4%) |
| Adjusted rate | 8.5% | 6.7% | 4.5% | 5.0% |
| Terminal rate | 3/38 (8%) | 3/34 (9%) | 2/30 (7%) | 1/23 (4%) |
| First incidence (days) | 551 | 729 (T) | 729 (T) | 705 |
| Poly-3 test | P=0.297N | P=0.529N | P=0.367N | P=0.412N |
| **Harderian Gland: Adenoma or Carcinoma** | | | | |
| Overall rate | 11/50 (22%) | 5/50 (10%) | 6/50 (12%) | 7/50 (14%) |
| Adjusted rate | 23.4% | 11.2% | 13.4% | 17.0% |
| Terminal rate | 10/38 (26%) | 5/34 (15%) | 5/30 (17%) | 3/23 (13%) |
| First incidence (days) | 551 | 729 (T) | 565 | 556 |
| Poly-3 test | P=0.322N | P=0.102N | P=0.168N | P=0.315N |
| **Liver: Hepatocellular Adenoma** | | | | |
| Overall rate | 34/50 (68%) | 33/50 (66%) | 37/50 (74%) | 35/50 (70%) |
| Adjusted rate | 70.6% | 69.9% | 77.9% | 79.5% |
| Terminal rate | 28/38 (74%) | 23/34 (68%) | 25/30 (83%) | 20/23 (87%) |
| First incidence (days) | 551 | 533 | 381 | 526 |
| Poly-3 test | P=0.135 | P=0.560N | P=0.276 | P=0.218 |
| **Liver: Hepatocellular Carcinoma** | | | | |
| Overall rate | 13/50 (26%) | 18/50 (36%) | 21/50 (42%) | 17/50 (34%) |
| Adjusted rate | 27.1% | 38.1% | 43.3% | 37.8% |
| Terminal rate | 7/38 (18%) | 9/34 (27%) | 7/30 (23%) | 4/23 (17%) |
| First incidence (days) | 583 | 453 | 381 | 391 |
| Poly-3 test | P=0.184 | P=0.177 | P=0.071 | P=0.190 |
| **Liver: Hepatocellular Adenoma or Carcinoma** | | | | |
| Overall rate | 40/50 (80%) | 42/50 (84%) | 43/50 (86%) | 41/50 (82%) |
| Adjusted rate | 81.0% | 85.8% | 87.2% | 87.1% |
| Terminal rate | 30/38 (79%) | 28/34 (82%) | 26/30 (87%) | 20/23 (87%) |
| First incidence (days) | 551 | 453 | 381 | 391 |
| Poly-3 test | P=0.250 | P=0.355 | P=0.284 | P=0.286 |
| **Liver: Hepatoblastoma** | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 4/50 (8%) | 3/50 (6%) |
| Adjusted rate | 2.1% | 2.2% | 9.0% | 7.4% |
| Terminal rate | 0/38 (0%) | 1/34 (3%) | 3/30 (10%) | 2/23 (9%) |
| First incidence (days) | 659 | 729 (T) | 702 | 610 |
| Poly-3 test | P=0.118 | P=0.750 | P=0.163 | P=0.256 |
| **Liver: Hepatocellular Carcinoma or Hepatoblastoma** | | | | |
| Overall rate | 14/50 (28%) | 18/50 (36%) | 24/50 (48%) | 20/50 (40%) |
| Adjusted rate | 29.1% | 38.1% | 49.4% | 44.0% |
| Terminal rate | 7/38 (18%) | 9/34 (27%) | 9/30 (30%) | 6/23 (26%) |
| First incidence (days) | 583 | 453 | 381 | 391 |
| Poly-3 test | P=0.071 | P=0.236 | P=0.030 | P=0.096 |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma** | | | | |
| Overall rate | 41/50 (82%) | 42/50 (84%) | 44/50 (88%) | 42/50 (84%) |
| Adjusted rate | 82.6% | 85.8% | 89.0% | 88.5% |
| Terminal rate | 30/38 (79%) | 28/34 (82%) | 26/30 (87%) | 20/23 (87%) |
| First incidence (days) | 551 | 453 | 381 | 391 |
| Poly-3 test | P=0.227 | P=0.437 | P=0.264 | P=0.291 |
| **Lung: Alveolar/bronchiolar Adenoma** | | | | |
| Overall rate | 13/50 (26%) | 31/50 (62%) | 31/50 (62%) | 29/50 (58%) |
| Adjusted rate | 27.5% | 66.7% | 66.9% | 67.9% |
| Terminal rate | 10/38 (26%) | 25/34 (74%) | 23/30 (77%) | 20/23 (87%) |
| First incidence (days) | 628 | 551 | 512 | 480 |
| Poly-3 test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| **Lung: Alveolar/bronchiolar Carcinoma** | | | | |
| Overall rate | 9/50 (18%) | 19/50 (38%) | 32/50 (64%) | 33/50 (66%) |
| Adjusted rate | 19.1% | 41.5% | 70.5% | 71.3% |
| Terminal rate | 6/38 (16%) | 15/34 (44%) | 25/30 (83%) | 12/23 (52%) |
| First incidence (days) | 631 | 551 | 565 | 420 |
| Poly-3 test | P<0.001 | P=0.014 | P<0.001 | P<0.001 |
| **Lung: Alveolar/bronchiolar Adenoma or Carcinoma** | | | | |
| Overall rate | 19/50 (38%) | 38/50 (76%) | 42/50 (84%) | 43/50 (86%) |
| Adjusted rate | 39.8% | 81.4% | 89.5% | 92.1% |
| Terminal rate | 14/38 (37%) | 31/34 (91%) | 30/30 (100%) | 21/23 (91%) |
| First incidence (days) | 628 | 551 | 512 | 420 |
| Poly-3 test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| **Skin: Fibrous Histiocytoma** | | | | |
| Overall rate | 1/50 (2%) | 2/50 (4%) | 1/50 (2%) | 3/50 (6%) |
| Adjusted rate | 2.2% | 4.5% | 2.3% | 7.4% |
| Terminal rate | 1/38 (3%) | 2/34 (6%) | 1/30 (3%) | 2/23 (9%) |
| First incidence (days) | 729 (T) | 729 (T) | 729 (T) | 609 |
| Poly-3 test | P=0.199 | P=0.486 | P=0.749 | P=0.258 |
| **Skin: Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma** | | | | |
| Overall rate | 2/50 (4%) | 3/50 (6%) | 1/50 (2%) | 3/50 (6%) |
| Adjusted rate | 4.3% | 6.7% | 2.3% | 7.4% |
| Terminal rate | 1/38 (3%) | 2/34 (6%) | 1/30 (3%) | 2/23 (9%) |
| First incidence (days) | 583 | 652 | 729 (T) | 609 |
| Poly-3 test | P=0.416 | P=0.479 | P=0.521N | P=0.433 |
| **Spleen: Hemangiosarcoma** | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 0/49 (0%) | 4/50 (8%) |
| Adjusted rate | 0.0% | 0.0% | 0.0% | 9.9% |
| Terminal rate | 0/38 (0%) | 0/34 (0%) | 0/30 (0%) | 3/23 (13%) |
| First incidence (days) | —[e] | —[f] | — | 556 |
| Poly-3 test | P=0.002 | — | — | P=0.045 |
| **Thyroid Gland (Follicular Cell): Adenoma** | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 0/49 (0%) | 3/50 (6%) |
| Adjusted rate | 0.0% | 0.0% | 0.0% | 7.5% |
| Terminal rate | 0/38 (0%) | 0/34 (0%) | 0/30 (0%) | 2/23 (9%) |
| First incidence (days) | — | — | — | 680 |
| Poly-3 test | P=0.010 | — | — | P=0.095 |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **All Organs: Hemangiosarcoma** | | | | |
| Overall rate | 0/50 (0%) | 1/50 (2%) | 2/50 (4%) | 4/50 (8%) |
| Adjusted rate | 0.0% | 2.2% | 4.5% | 9.9% |
| Terminal rate | 0/38 (0%) | 0/34 (0%) | 2/30 (7%) | 3/23 (13%) |
| First incidence (days) | — | 654 | 729 (T) | 556 |
| Poly-3 test | P=0.015 | P=0.493 | P=0.226 | P=0.045 |
| **All Organs: Hemangioma or Hemangiosarcoma** | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 2/50 (4%) | 4/50 (8%) |
| Adjusted rate | 2.2% | 2.2% | 4.5% | 9.9% |
| Terminal rate | 1/38 (3%) | 0/34 (0%) | 2/30 (7%) | 3/23 (13%) |
| First incidence (days) | 729 (T) | 654 | 729 (T) | 556 |
| Poly-3 test | P=0.054 | P=0.753 | P=0.482 | P=0.141 |
| **All Organs: Benign Neoplasms** | | | | |
| Overall rate | 43/50 (86%) | 45/50 (90%) | 46/50 (92%) | 44/50 (88%) |
| Adjusted rate | 87.8% | 93.2% | 94.5% | 95.6% |
| Terminal rate | 34/38 (90%) | 32/34 (94%) | 30/30 (100%) | 23/23 (100%) |
| First incidence (days) | 551 | 533 | 381 | 480 |
| Poly-3 test | P=0.091 | P=0.275 | P=0.192 | P=0.132 |
| **All Organs: Malignant Neoplasms** | | | | |
| Overall rate | 27/50 (54%) | 34/50 (68%) | 47/50 (94%) | 45/50 (90%) |
| Adjusted rate | 55.0% | 69.8% | 95.2% | 90.8% |
| Terminal rate | 18/38 (47%) | 21/34 (62%) | 28/30 (93%) | 19/23 (83%) |
| First incidence (days) | 551 | 453 | 381 | 391 |
| Poly-3 test | P<0.001 | P=0.094 | P<0.001 | P<0.001 |
| **All Organs: Benign or Malignant Neoplasms** | | | | |
| Overall rate | 48/50 (96%) | 50/50 (100%) | 50/50 (100%) | 50/50 (100%) |
| Adjusted rate | 96.0% | 100% | 100% | 100% |
| Terminal rate | 36/38 (95%) | 34/34 (100%) | 30/30 (100%) | 23/23 (100%) |
| First incidence (days) | 551 | 453 | 381 | 391 |
| Poly-3 test | P=0.114 | P=0.237 | P=0.237 | P=0.237 |

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, spleen, 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 are the P values 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 C3a**
**Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Male B6C3F1 Mice[a]**

| Study | Incidence in Controls | | |
|---|---|---|---|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| **Historical Incidence: Inhalation Studies** | | | |
| Cumene | 13/50 | 9/50 | 19/50 |
| Decalin | 8/50 | 8/50 | 15/50 |
| Divinylbenzene | 12/49 | 5/49 | 16/49 |
| Methyl isobutyl ketone | 9/50 | 5/50 | 14/50 |
| α-Methylstyrene | 8/50 | 5/50 | 13/50 |
| Propargyl alcohol | 10/50 | 7/50 | 17/50 |
| Propylene glycol mono-*t*-butyl ether | 13/50 | 6/50 | 17/50 |
| Stoddard solvent IIC | 6/50 | 7/50 | 13/50 |
| Vanadium pentoxide | 13/50 | 12/50 | 22/50 |
| **Overall Historical Incidence: Inhalation Studies** | | | |
| Total (%) | 92/449 (20.5%) | 64/449 (14.3%) | 146/449 (32.5%) |
| Mean ± standard deviation | 20.5% ± 5.3% | 14.2% ± 4.6% | 32.5% ± 5.9% |
| Range | 12%-26% | 10%-24% | 26%-44% |
| **Overall Historical Incidence: All Routes** | | | |
| Total (%) | 263/1,498 (17.6%) | 161/1,498 (10.8%) | 401/1,498 (26.8%) |
| Mean ± standard deviation | 17.9% ± 6.1% | 10.9% ± 5.6% | 27.2% ± 7.8% |
| Range | 6%-28% | 2%-24% | 12%-44% |

[a] Data as of March 2, 2007

**TABLE C3b**
**Historical Incidence of Hepatocellular Neoplasms in Control Male B6C3F1 Mice[a]**

| Study | Incidence in Controls | | |
|---|---|---|---|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| **Historical Incidence: Inhalation Studies** | | | |
| Cumene | 34/50 | 13/50 | 40/50 |
| Decalin | 22/50 | 10/50 | 28/50 |
| Divinylbenzene | 22/50 | 13/50 | 30/50 |
| Methyl isobutyl ketone | 17/50 | 12/50 | 27/50 |
| α-Methylstyrene | 24/50 | 10/50 | 28/50 |
| Propargyl alcohol | 21/49 | 10/49 | 26/49 |
| Propylene glycol mono-*t*-butyl ether | 18/50 | 9/50 | 25/50 |
| Stoddard solvent IIC | 23/50 | 16/50 | 34/50 |
| Vanadium pentoxide | 15/50 | 14/50 | 26/50 |
| **Overall Historical Incidence: Inhalation Studies** | | | |
| Total (%) | 196/449 (43.7%) | 107/449 (23.8%) | 264/449 (58.8%) |
| Mean ± standard deviation | 43.7% ± 10.9% | 23.8% ± 4.6% | 58.8% ± 9.6% |
| Range | 30%-68% | 18%-32% | 50%-80% |
| **Overall Historical Incidence: All Routes** | | | |
| Total (%) | 633/1,496 (42.3%) | 382/1,496 (25.5%) | 874/1,496 (58.4%) |
| Mean ± standard deviation | 43.3% ± 14.2% | 26.0% ± 9.1% | 59.6% ± 15.4% |
| Range | 14%-70% | 8%-48% | 20%-85% |

[a] Data as of March 2, 2007

**TABLE C3c**
**Historical Incidence of Hemangiosarcoma in Control Male B6C3F1 Mice[a]**

| Study | Incidence in Controls | |
|---|---|---|
| | Spleen | All Organs |
| **Historical Incidence: Inhalation Studies** | | |
| Cumene | 0/50 | 0/50 |
| Decalin | 0/49 | 1/50 |
| Divinylbenzene | 1/49 | 6/50 |
| Methyl isobutyl ketone | 1/50 | 3/50 |
| α-Methylstyrene | 1/50 | 4/50 |
| Propargyl alcohol | 1/49 | 2/50 |
| Propylene glycol mono-*t*-butyl ether | 2/48 | 3/50 |
| Stoddard solvent IIC | 0/49 | 1/50 |
| Vanadium pentoxide | 0/50 | 1/50 |
| **Overall Historical Incidence: Inhalation Studies** | | |
| Total (%) | 6/444 (1.4%) | 21/450 (4.7%) |
| Mean ± standard deviation | 1.4% ± 1.5% | 4.7% ± 3.7% |
| Range | 0%-4% | 0%-12% |
| **Overall Historical Incidence: All Routes** | | |
| Total (%) | 24/1,483 (1.6%) | 76/1,499 (5.1%) |
| Mean ± standard deviation | 1.7% ± 1.2% | 5.2% ± 3.2% |
| Range | 0%-4% | 0%-12% |

[a] Data as of March 2, 2007

**TABLE C3d**
**Historical Incidence of Follicular Cell Adenoma of the Thyroid Gland in Control Male B6C3F1 Mice[a]**

| Study | Incidence in Controls |
|---|---|
| **Historical Incidence: Inhalation Studies** | |
| Cumene | 0/50 |
| Decalin | 0/48 |
| Divinylbenzene | 0/49 |
| Methyl isobutyl ketone | 0/50 |
| α-Methylstyrene | 3/50 |
| Propargyl alcohol | 0/49 |
| Propylene glycol mono-*t*-butyl ether | 1/48 |
| Stoddard solvent IIC | 1/49 |
| Vanadium pentoxide | 0/48 |
| **Overall Historical Incidence: Inhalation Studies** | |
| Total (%) | 5/441 (1.1%) |
| Mean ± standard deviation | 1.1% ± 2.0% |
| Range | 0%-6% |
| **Overall Historical Incidence: All Routes** | |
| Total (%) | 21/1,483 (1.4%) |
| Mean ± standard deviation | 1.4% ± 1.8% |
| Range | 0%-6% |

[a] Data as of March 2, 2007

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 7 | 9 | 13 | 20 |
| Natural deaths | 5 | 7 | 7 | 7 |
| Survivors | | | | |
| Terminal sacrifice | 38 | 34 | 30 | 23 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Gallbladder | (43) | (32) | (37) | (38) |
| Degeneration, hyaline | 1 (2%) | | | |
| Hyperplasia | 1 (2%) | | | |
| Intestine large, cecum | (49) | (47) | (44) | (44) |
| Infiltration cellular, histiocyte | | | | 1 (2%) |
| Inflammation, acute | | | | 1 (2%) |
| Intestine large, colon | (50) | (46) | (45) | (48) |
| Intestine small, duodenum | (49) | (44) | (43) | (44) |
| Intestine small, ileum | (49) | (46) | (43) | (44) |
| Necrosis | | | 1 (2%) | |
| Peyer's patch, inflammation, acute | 1 (2%) | | | |
| Intestine small, jejunum | (48) | (46) | (43) | (44) |
| Peyer's patch, hyperplasia | | 1 (2%) | | |
| Liver | (50) | (50) | (50) | (50) |
| Angiectasis | 1 (2%) | 3 (6%) | 1 (2%) | |
| Basophilic focus | 7 (14%) | 5 (10%) | 4 (8%) | 5 (10%) |
| Clear cell focus | 24 (48%) | 11 (22%) | 20 (40%) | 11 (22%) |
| Eosinophilic focus | 6 (12%) | 5 (10%) | 16 (32%) | 14 (28%) |
| Hepatodiaphragmatic nodule | | | | 1 (2%) |
| Inflammation, granulomatous | 1 (2%) | | 1 (2%) | |
| Mixed cell focus | 2 (4%) | 3 (6%) | 1 (2%) | 1 (2%) |
| Necrosis | 2 (4%) | 4 (8%) | 4 (8%) | 1 (2%) |
| Tension lipidosis | 1 (2%) | 1 (2%) | 2 (4%) | |
| Centrilobular, necrosis | 1 (2%) | 4 (8%) | | |
| Mesentery | (3) | (7) | (6) | (3) |
| Thrombosis | 1 (33%) | | | |
| Fat, necrosis | 3 (100%) | 6 (86%) | 6 (100%) | 3 (100%) |
| Pancreas | (50) | (50) | (49) | (49) |
| Atrophy | | 1 (2%) | | |
| Duct, cyst | 1 (2%) | | | |
| Stomach, forestomach | (50) | (50) | (50) | (49) |
| Inflammation | | 2 (4%) | 1 (2%) | 5 (10%) |
| Ulcer | 1 (2%) | 4 (8%) | 6 (12%) | 6 (12%) |
| Epithelium, erosion | | 1 (2%) | | 1 (2%) |
| Epithelium, hyperplasia | 2 (4%) | 7 (14%) | 8 (16%) | 13 (27%) |
| Stomach, glandular | (50) | (48) | (48) | (48) |
| Mineralization | | 1 (2%) | | |
| Necrosis | | 2 (4%) | | 1 (2%) |
| Tongue | (1) | | | |
| Tooth | (15) | (8) | (8) | (6) |
| Malformation | 14 (93%) | 7 (88%) | 8 (100%) | 6 (100%) |

[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 Cumene**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Cardiovascular System** | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Cardiomyopathy | 10 (20%) | 18 (36%) | 13 (26%) | 17 (34%) |
| Inflammation, acute | | | | 2 (4%) |
| Mineralization | | 1 (2%) | | 1 (2%) |
| Necrosis | | | | 1 (2%) |
| Thrombosis | 1 (2%) | | 1 (2%) | 2 (4%) |
| Artery, inflammation, chronic active | 1 (2%) | | 1 (2%) | 1 (2%) |
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (49) | (50) |
| Atrophy | | | | 1 (2%) |
| Degeneration | | 1 (2%) | | |
| Hyperplasia | 14 (28%) | 15 (30%) | 12 (24%) | 8 (16%) |
| Hypertrophy | 29 (58%) | 16 (32%) | 14 (29%) | 9 (18%) |
| Adrenal medulla | (50) | (50) | (48) | (50) |
| Hyperplasia | 2 (4%) | 1 (2%) | | |
| Islets, pancreatic | (50) | (50) | (49) | (49) |
| Hyperplasia | 1 (2%) | 3 (6%) | 1 (2%) | |
| Hypertrophy | | | 1 (2%) | |
| Pituitary gland | (48) | (49) | (49) | (49) |
| Pars distalis, hyperplasia | 1 (2%) | 1 (2%) | 3 (6%) | 1 (2%) |
| Thyroid gland | (50) | (50) | (49) | (50) |
| Follicular cell, hyperplasia | 7 (14%) | 7 (14%) | 7 (14%) | 11 (22%) |
| **General Body System** | | | | |
| None | | | | |
| **Genital System** | | | | |
| Epididymis | (50) | (50) | (49) | (50) |
| Angiectasis | | 1 (2%) | | |
| Granuloma sperm | | 2 (4%) | | 1 (2%) |
| Preputial gland | (49) | (50) | (50) | (50) |
| Ectasia | | 1 (2%) | 1 (2%) | |
| Inflammation, chronic active | 1 (2%) | | | |
| Prostate | (50) | (49) | (48) | (49) |
| Angiectasis | 1 (2%) | | | |
| Hyperplasia | | 1 (2%) | | 1 (2%) |
| Infiltration cellular, polymorphonuclear | | | | 1 (2%) |
| Artery, inflammation, chronic active | 1 (2%) | | | |
| Testes | (50) | (50) | (49) | (50) |
| Atrophy | 5 (10%) | 3 (6%) | 1 (2%) | 1 (2%) |
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (49) | (49) |
| Thrombosis | | 1 (2%) | | |
| Lymph node | | | (1) | (2) |
| Iliac, infiltration cellular, mixed cell | | | 1 (100%) | |
| Lymph node, bronchial | (45) | (41) | (45) | (35) |
| Lymph node, mandibular | (34) | (35) | (22) | (25) |
| Infiltration cellular, plasma cell | 1 (3%) | | | |
| Lymph node, mediastinal | (37) | (42) | (40) | (39) |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Hematopoietic System** (continued) | | | | |
| Lymph node, mesenteric | (48) | (47) | (46) | (44) |
| Infiltration cellular, mixed cell | | | 1 (2%) | |
| Infiltration cellular, plasma cell | 1 (2%) | | 1 (2%) | |
| Spleen | (50) | (50) | (49) | (50) |
| Hematopoietic cell proliferation | 3 (6%) | 1 (2%) | 2 (4%) | |
| Infiltration cellular, plasma cell | 1 (2%) | | | |
| Thymus | (45) | (44) | (38) | (44) |
| Artery, inflammation | | | 1 (3%) | |
| **Integumentary System** | | | | |
| Skin | (50) | (50) | (49) | (50) |
| Cyst epithelial inclusion | | 1 (2%) | | |
| Inflammation, acute | | | | 1 (2%) |
| Inflammation, chronic active | 1 (2%) | 2 (4%) | 6 (12%) | 3 (6%) |
| Ulcer | | | | 1 (2%) |
| Epidermis, abscess | | | | 1 (2%) |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Hyperostosis | 1 (2%) | | | |
| Skeletal muscle | | | (1) | |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Degeneration | | 1 (2%) | | |
| Gliosis | | | | 1 (2%) |
| Necrosis | | | 1 (2%) | |
| Ventricle, infiltration cellular, polymorphonuclear | | | | 1 (2%) |
| **Respiratory System** | | | | |
| Larynx | (50) | (49) | (50) | (50) |
| Metaplasia, squamous | | | | 1 (2%) |
| Lung | (50) | (50) | (50) | (50) |
| Thrombosis | | 1 (2%) | 1 (2%) | 1 (2%) |
| Alveolar epithelium, bronchiole, metaplasia | 5 (10%) | 43 (86%) | 42 (84%) | 39 (78%) |
| Alveolar epithelium, hyperplasia | 3 (6%) | 3 (6%) | 4 (8%) | |
| Alveolus, infiltration cellular, histiocyte | 2 (4%) | 3 (6%) | | 1 (2%) |
| Bronchiole, hyperplasia | | 11 (22%) | 17 (34%) | 18 (36%) |
| Bronchus, inflammation, acute | | | 1 (2%) | |
| Bronchus, necrosis | | | | 1 (2%) |
| Nose | (50) | (50) | (49) | (48) |
| Inflammation, suppurative | 2 (4%) | 2 (4%) | 9 (18%) | 6 (13%) |
| Polyp, inflammatory | 1 (2%) | | | |
| Glands, olfactory epithelium, hyperplasia | 3 (6%) | 11 (22%) | 9 (18%) | 23 (48%) |
| Olfactory epithelium, accumulation, hyaline droplet | 1 (2%) | | | |
| Olfactory epithelium, atrophy | 4 (8%) | 13 (26%) | 11 (22%) | 38 (79%) |
| Olfactory epithelium, hyperplasia, atypical | | | 5 (10%) | 11 (23%) |
| Olfactory epithelium, hyperplasia, basal cell | | | 15 (31%) | 33 (69%) |
| Respiratory epithelium, accumulation, hyaline droplet | 1 (2%) | | | |
| Respiratory epithelium, metaplasia, squamous | 1 (2%) | 1 (2%) | 1 (2%) | 5 (10%) |
| Vomeronasal organ, inflammation, suppurative | | | 1 (2%) | |

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Respiratory System** (continued) | | | | |
| Pleura | | | | (2) |
| Trachea | (50) | (50) | (49) | (50) |
| Necrosis | | | | 1 (2%) |
| **Special Senses System** | | | | |
| Eye | (49) | (50) | (49) | (48) |
| Cataract | 1 (2%) | 1 (2%) | | 1 (2%) |
| Cornea, inflammation, chronic active | | 2 (4%) | 1 (2%) | 4 (8%) |
| Cornea, mineralization | | | | 1 (2%) |
| Harderian gland | (48) | (50) | (49) | (50) |
| Hyperplasia | 2 (4%) | 2 (4%) | 1 (2%) | 1 (2%) |
| Hypertrophy | | | | 1 (2%) |
| Inflammation, chronic | 1 (2%) | | | |
| Necrosis | | | | 1 (2%) |
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Infarct | 1 (2%) | 4 (8%) | 3 (6%) | 7 (14%) |
| Inflammation, suppurative | | | | 1 (2%) |
| Metaplasia, osseous | 3 (6%) | 1 (2%) | | 1 (2%) |
| Mineralization | 1 (2%) | 1 (2%) | | 2 (4%) |
| Nephropathy | 47 (94%) | 44 (88%) | 45 (90%) | 41 (82%) |
| Artery, inflammation, chronic active | 1 (2%) | | | 1 (2%) |
| Capsule, fibrosis | | 1 (2%) | | |
| Perirenal tissue, thrombosis | | | | 1 (2%) |
| Renal tubule, cyst | 1 (2%) | | 1 (2%) | |
| Renal tubule, hyperplasia | | 2 (4%) | | |
| Renal tubule, necrosis | | | | 1 (2%) |
| Renal tubule, pigmentation | | | | 1 (2%) |
| Urinary bladder | (50) | (48) | (49) | (48) |

# APPENDIX D
# SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF CUMENE

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

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 8 | 10 | 8 | 12 |
| Natural deaths | 5 | 4 | 3 | 3 |
| Survivors | | | | |
| Died last week of study | | | | 1 |
| Terminal sacrifice | 37 | 36 | 39 | 34 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Esophagus | (50) | (50) | (50) | (50) |
| Squamous cell carcinoma | | | | 1 (2%) |
| Gallbladder | (38) | (41) | (44) | (37) |
| Intestine large, cecum | (46) | (47) | (49) | (47) |
| Intestine large, colon | (47) | (50) | (50) | (48) |
| Intestine small, duodenum | (46) | (47) | (49) | (48) |
| Carcinoma | | 1 (2%) | | |
| Intestine small, ileum | (46) | (47) | (49) | (47) |
| Intestine small, jejunum | (46) | (47) | (49) | (47) |
| Liver | (50) | (50) | (50) | (50) |
| Hemangiosarcoma | | 1 (2%) | | |
| Hepatoblastoma | | | 1 (2%) | |
| Hepatocellular adenoma | 9 (18%) | 10 (20%) | 18 (36%) | 19 (38%) |
| Hepatocellular adenoma, multiple | 9 (18%) | 13 (26%) | 9 (18%) | 10 (20%) |
| Hepatocellular carcinoma | 8 (16%) | 6 (12%) | 4 (8%) | 12 (24%) |
| Hepatocellular carcinoma, multiple | 2 (4%) | 1 (2%) | 2 (4%) | |
| Hepatocholangiocarcinoma | | 1 (2%) | | |
| Ito cell tumor benign | | | | 1 (2%) |
| Mesentery | (11) | (12) | (11) | (9) |
| Hemangiosarcoma | | | 1 (9%) | |
| Sarcoma | | 1 (8%) | | |
| Pancreas | (49) | (50) | (50) | (50) |
| Carcinoma, metastatic, uncertain primary site | 1 (2%) | | | |
| Salivary glands | (50) | (50) | (50) | (50) |
| Stomach, forestomach | (49) | (50) | (50) | (50) |
| Squamous cell papilloma | | 1 (2%) | 2 (4%) | 2 (4%) |
| Epithelium, squamous cell carcinoma | 1 (2%) | | | |
| Stomach, glandular | (49) | (50) | (50) | (49) |
| **Cardiovascular System** | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (2%) |
| Carcinoma, metastatic, uncertain primary site | 1 (2%) | | | 1 (2%) |
| Hepatocholangiocarcinoma, metastatic, liver | | 1 (2%) | | |
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (50) | (49) |
| Hepatocholangiocarcinoma, metastatic, liver | | 1 (2%) | | |
| Adrenal medulla | (50) | (49) | (50) | (49) |
| Pheochromocytoma malignant | | | 2 (4%) | 1 (2%) |

**TABLE D1**
**Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Endocrine System** (continued) | | | | |
| Islets, pancreatic | (49) | (50) | (50) | (50) |
| Adenoma | 1 (2%) | | | |
| Carcinoma | | 1 (2%) | 1 (2%) | 1 (2%) |
| Pituitary gland | (50) | (49) | (50) | (48) |
| Pars distalis, adenoma | 8 (16%) | 6 (12%) | 6 (12%) | 10 (21%) |
| Pars intermedia, adenoma | 2 (4%) | | 1 (2%) | |
| Thyroid gland | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Bilateral, follicular cell, carcinoma | | 1 (2%) | | |
| C-cell, adenoma | | 1 (2%) | | |
| Follicular cell, adenoma | 1 (2%) | 4 (8%) | | 3 (6%) |
| Follicular cell, carcinoma | 1 (2%) | | | |
| **General Body System** | | | | |
| None | | | | |
| **Genital System** | | | | |
| Ovary | (48) | (50) | (50) | (49) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Cystadenocarcinoma | | 1 (2%) | 1 (2%) | |
| Cystadenoma | 5 (10%) | 2 (4%) | | 3 (6%) |
| Luteoma | | | 1 (2%) | |
| Teratoma benign | 1 (2%) | | | |
| Yolk sac carcinoma | | 1 (2%) | | |
| Uterus | (50) | (50) | (50) | (50) |
| Leiomyoma | | | 1 (2%) | |
| Polyp stromal | 3 (6%) | | 1 (2%) | 2 (4%) |
| Bilateral, polyp stromal | | | 1 (2%) | |
| Endometrium, carcinoma | 3 (6%) | | 1 (2%) | |
| **Hematopoietic System** | | | | |
| Bone marrow | (49) | (50) | (50) | (50) |
| Hemangiosarcoma | | | 2 (4%) | |
| Lymph node | (4) | (9) | (5) | (5) |
| Axillary, hemangiosarcoma, metastatic, spleen | | | 1 (20%) | |
| Renal, alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (20%) | |
| Renal, hepatocholangiocarcinoma, metastatic, liver | | 1 (11%) | | |
| Lymph node, bronchial | (39) | (36) | (40) | (46) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (2%) |
| Carcinoma, metastatic, uncertain primary site | 1 (3%) | | | |
| Lymph node, mandibular | (35) | (42) | (38) | (42) |
| Lymph node, mediastinal | (44) | (40) | (42) | (40) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (2%) | 2 (5%) |
| Carcinoma, metastatic, uncertain primary site | 1 (2%) | | | |
| Hepatocholangiocarcinoma, metastatic, liver | | 1 (3%) | | |
| Lymph node, mesenteric | (46) | (46) | (48) | (48) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (2%) |
| Spleen | (49) | (50) | (50) | (50) |
| Hemangiosarcoma | | | 3 (6%) | 1 (2%) |

**TABLE D1**
**Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Hematopoietic System** (continued) | | | | |
| Thymus | (49) | (50) | (48) | (48) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (2%) |
| Carcinoma, metastatic, uncertain primary site | 1 (2%) | | | |
| Hepatocholangiocarcinoma, metastatic, liver | | 1 (2%) | | |
| **Integumentary System** | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Carcinoma | 1 (2%) | 1 (2%) | | |
| Fibroadenoma | | | | 1 (2%) |
| Skin | (50) | (50) | (50) | (50) |
| Basal cell carcinoma | | | 1 (2%) | |
| Squamous cell carcinoma | 1 (2%) | | | |
| Subcutaneous tissue, fibrous histiocytoma | | | 1 (2%) | |
| Subcutaneous tissue, hemangioma | | | | 1 (2%) |
| Subcutaneous tissue, hemangiosarcoma | 1 (2%) | 2 (4%) | 1 (2%) | |
| Subcutaneous tissue, sarcoma | | 1 (2%) | 1 (2%) | 2 (4%) |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Hemangiosarcoma | | | 1 (2%) | |
| Skeletal muscle | (1) | (1) | (1) | |
| Carcinoma, metastatic, uncertain primary site | 1 (100%) | | | |
| Hemangiosarcoma | | | 1 (100%) | |
| Hepatocholangiocarcinoma, metastatic, liver | | 1 (100%) | | |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (2%) |
| **Respiratory System** | | | | |
| Larynx | (50) | (49) | (50) | (50) |
| Squamous cell papilloma | | | | 1 (2%) |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | 1 (2%) | 13 (26%) | 16 (32%) | 8 (16%) |
| Alveolar/bronchiolar adenoma, multiple | | 13 (26%) | 20 (40%) | 30 (60%) |
| Alveolar/bronchiolar carcinoma | 3 (6%) | 10 (20%) | 13 (26%) | 15 (30%) |
| Alveolar/bronchiolar carcinoma, multiple | | 6 (12%) | 7 (14%) | 19 (38%) |
| Carcinoma, metastatic, Harderian gland | 3 (6%) | | | |
| Carcinoma, metastatic, uncertain primary site | 1 (2%) | | | 1 (2%) |
| Hepatocellular carcinoma, metastatic, liver | 4 (8%) | 5 (10%) | 2 (4%) | 3 (6%) |
| Hepatocholangiocarcinoma, metastatic, liver | | 1 (2%) | | |
| Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (2%) |
| Nose | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, Harderian gland | 1 (2%) | | | |
| Hemangioma | | | | 1 (2%) |
| Hemangiosarcoma | | | 1 (2%) | |
| Respiratory epithelium, adenoma | | 1 (2%) | | |

**TABLE D1**
**Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Respiratory System** (continued) | | | | |
| Pleura | | (1) | (1) | (2) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (100%) | 1 (50%) |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (50%) |
| Hepatocholangiocarcinoma, metastatic, liver | | 1 (100%) | | |
| Trachea | (50) | (50) | (50) | (50) |
| **Special Senses System** | | | | |
| Eye | (49) | (50) | (50) | (49) |
| Harderian gland | (50) | (49) | (50) | (50) |
| Adenoma | 8 (16%) | 4 (8%) | | 2 (4%) |
| Carcinoma | 5 (10%) | 1 (2%) | 1 (2%) | 5 (10%) |
| **Urinary System** | | | | |
| Kidney | (49) | (50) | (50) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (2%) |
| Carcinoma, metastatic, uncertain primary site | 1 (2%) | | | 1 (2%) |
| Hepatocholangiocarcinoma, metastatic, liver | | 1 (2%) | | |
| Urinary bladder | (48) | (48) | (49) | (48) |
| Transitional epithelium, carcinoma | | | 1 (2%) | |
| **Systemic Lesions** | | | | |
| Multiple organs[b] | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | | 1 (2%) | 2 (4%) | |
| Lymphoma malignant | 7 (14%) | 15 (30%) | 6 (12%) | 12 (24%) |
| **Neoplasm Summary** | | | | |
| Total animals with primary neoplasms[c] | 42 | 44 | 50 | 50 |
| Total primary neoplasms | 81 | 120 | 131 | 163 |
| Total animals with benign neoplasms | 34 | 39 | 47 | 47 |
| Total benign neoplasms | 48 | 68 | 76 | 94 |
| Total animals with malignant neoplasms | 26 | 33 | 37 | 43 |
| Total malignant neoplasms | 33 | 52 | 55 | 69 |
| Total animals with metastatic neoplasms | 7 | 6 | 3 | 7 |
| Total metastatic neoplasms | 16 | 14 | 6 | 19 |
| Total animals with malignant neoplasms-uncertain primary site | 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 D2**
**Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Harderian Gland: Adenoma** | | | | |
| Overall rate[a] | 8/50 (16%) | 4/50 (8%) | 0/50 (0%) | 2/50 (4%) |
| Adjusted rate[b] | 18.0% | 8.9% | 0.0% | 4.3% |
| Terminal rate[c] | 7/37 (19%) | 3/36 (8%) | 0/39 (0%) | 2/35 (6%) |
| First incidence (days) | 656 | 726 | —[e] | 731 (T) |
| Poly-3 test[d] | P=0.012N | P=0.171N | P=0.003N | P=0.036N |
| **Harderian Gland: Carcinoma** | | | | |
| Overall rate | 5/50 (10%) | 1/50 (2%) | 1/50 (2%) | 5/50 (10%) |
| Adjusted rate | 11.2% | 2.2% | 2.1% | 10.6% |
| Terminal rate | 3/37 (8%) | 1/36 (3%) | 1/39 (3%) | 3/35 (9%) |
| First incidence (days) | 683 | 731 (T) | 731 (T) | 672 |
| Poly-3 test | P=0.441 | P=0.099N | P=0.090N | P=0.591N |
| **Harderian Gland: Adenoma or Carcinoma** | | | | |
| Overall rate | 12/50 (24%) | 5/50 (10%) | 1/50 (2%) | 6/50 (12%) |
| Adjusted rate | 26.8% | 11.2% | 2.1% | 12.7% |
| Terminal rate | 9/37 (24%) | 4/36 (11%) | 1/39 (3%) | 4/35 (11%) |
| First incidence (days) | 656 | 726 | 731 (T) | 672 |
| Poly-3 test | P=0.056N | P=0.050N | P<0.001N | P=0.073N |
| **Liver: Hepatocellular Adenoma** | | | | |
| Overall rate | 18/50 (36%) | 23/50 (46%) | 27/50 (54%)[f] | 29/50 (58%) |
| Adjusted rate | 40.5% | 50.0% | 56.4% | 59.8% |
| Terminal rate | 17/37 (46%) | 17/36 (47%) | 22/39 (56%) | 19/35 (54%) |
| First incidence (days) | 654 | 609 | 618 | 662 |
| Poly-3 test | P=0.040 | P=0.243 | P=0.091 | P=0.046 |
| **Liver: Hepatocellular Carcinoma** | | | | |
| Overall rate | 10/50 (20%) | 7/50 (14%) | 6/50 (12%) | 12/50 (24%) |
| Adjusted rate | 22.2% | 15.5% | 12.7% | 25.4% |
| Terminal rate | 7/37 (19%) | 5/36 (14%) | 4/39 (10%) | 10/35 (29%) |
| First incidence (days) | 607 | 623 | 651 | 702 |
| Poly-3 test | P=0.311 | P=0.291N | P=0.177N | P=0.455 |
| **Liver: Hepatocellular Adenoma or Carcinoma** | | | | |
| Overall rate | 25/50 (50%) | 26/50 (52%) | 29/50 (58%)[f] | 36/50 (72%) |
| Adjusted rate | 55.6% | 56.5% | 60.4% | 74.1% |
| Terminal rate | 22/37 (60%) | 20/36 (56%) | 23/39 (59%) | 25/35 (71%) |
| First incidence (days) | 607 | 609 | 618 | 662 |
| Poly-3 test | P=0.024 | P=0.549 | P=0.395 | P=0.043 |
| **Liver: Hepatocellular Carcinoma or Hepatoblastoma** | | | | |
| Overall rate | 10/50 (20%) | 7/50 (14%) | 7/50 (14%) | 12/50 (24%) |
| Adjusted rate | 22.2% | 15.5% | 14.7% | 25.4% |
| Terminal rate | 7/37 (19%) | 5/36 (14%) | 4/39 (10%) | 10/35 (29%) |
| First incidence (days) | 607 | 623 | 618 | 702 |
| Poly-3 test | P=0.305 | P=0.291N | P=0.255N | P=0.455 |
| **Lung: Alveolar/bronchiolar Adenoma** | | | | |
| Overall rate | 1/50 (2%) | 26/50 (52%) | 36/50 (72%) | 38/50 (76%) |
| Adjusted rate | 2.3% | 56.3% | 74.5% | 77.9% |
| Terminal rate | 1/37 (3%) | 21/36 (58%) | 31/39 (80%) | 29/35 (83%) |
| First incidence (days) | 731 (T) | 555 | 495 | 565 |
| Poly-3 test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |

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

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Lung: Alveolar/bronchiolar Carcinoma** | | | | |
| Overall rate | 3/50 (6%) | 16/50 (32%) | 20/50 (40%) | 34/50 (68%) |
| Adjusted rate | 6.7% | 35.3% | 41.9% | 69.5% |
| Terminal rate | 2/37 (5%) | 13/36 (36%) | 15/39 (39%) | 24/35 (69%) |
| First incidence (days) | 533 | 646 | 618 | 513 |
| Poly-3 test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| **Lung: Alveolar/bronchiolar Adenoma or Carcinoma** | | | | |
| Overall rate | 4/50 (8%) | 31/50 (62%) | 42/50 (84%) | 46/50 (92%) |
| Adjusted rate | 8.9% | 66.8% | 86.0% | 92.4% |
| Terminal rate | 3/37 (8%) | 25/36 (69%) | 34/39 (87%) | 33/35 (94%) |
| First incidence (days) | 533 | 555 | 495 | 513 |
| Poly-3 test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| **Ovary: Cystadenoma** | | | | |
| Overall rate | 5/48 (10%) | 2/50 (4%) | 0/50 (0%) | 3/49 (6%) |
| Adjusted rate | 11.5% | 4.5% | 0.0% | 6.5% |
| Terminal rate | 5/37 (14%) | 2/36 (6%) | 0/39 (0%) | 3/34 (9%) |
| First incidence (days) | 731 (T) | 731 (T) | — | 731 (T) |
| Poly-3 test | P=0.274N | P=0.204N | P=0.025N | P=0.326N |
| **Pituitary Gland (Pars Distalis): Adenoma** | | | | |
| Overall rate | 8/50 (16%) | 6/49 (12%) | 6/50 (12%) | 10/48 (21%) |
| Adjusted rate | 18.1% | 13.6% | 12.8% | 21.8% |
| Terminal rate | 8/37 (22%) | 6/36 (17%) | 5/39 (13%) | 8/34 (24%) |
| First incidence (days) | 731 (T) | 731 (T) | 709 | 565 |
| Poly-3 test | P=0.299 | P=0.385N | P=0.342N | P=0.433 |
| **Spleen: Hemangiosarcoma** | | | | |
| Overall rate | 0/49 (0%) | 0/50 (0%) | 3/50 (6%) | 1/50 (2%) |
| Adjusted rate | 0.0% | 0.0% | 6.4% | 2.1% |
| Terminal rate | 0/37 (0%) | 0/36 (0%) | 2/39 (5%) | 1/35 (3%) |
| First incidence (days) | — | — | 673 | 731 (T) |
| Poly-3 test | P=0.271 | —[g] | P=0.130 | P=0.513 |
| **Thyroid Gland (Follicular Cell): Adenoma** | | | | |
| Overall rate | 1/50 (2%) | 4/50 (8%) | 0/50 (0%) | 3/50 (6%) |
| Adjusted rate | 2.3% | 8.9% | 0.0% | 6.4% |
| Terminal rate | 1/37 (3%) | 4/36 (11%) | 0/39 (0%) | 3/35 (9%) |
| First incidence (days) | 731 (T) | 731 (T) | — | 731 (T) |
| Poly-3 test | P=0.432 | P=0.183 | P=0.489N | P=0.329 |
| **Thyroid Gland (Follicular Cell): Adenoma or Carcinoma** | | | | |
| Overall rate | 2/50 (4%) | 5/50 (10%) | 0/50 (0%) | 3/50 (6%) |
| Adjusted rate | 4.5% | 11.2% | 0.0% | 6.4% |
| Terminal rate | 1/37 (3%) | 5/36 (14%) | 0/39 (0%) | 3/35 (9%) |
| First incidence (days) | 683 | 731 (T) | — | 731 (T) |
| Poly-3 test | P=0.519N | P=0.220 | P=0.226N | P=0.527 |

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

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Uterus: Carcinoma** | | | | |
| Overall rate | 3/50 (6%) | 0/50 (0%) | 1/50 (2%) | 0/50 (0%) |
| Adjusted rate | 6.8% | 0.0% | 2.1% | 0.0% |
| Terminal rate | 3/37 (8%) | 0/36 (0%) | 1/39 (3%) | 0/35 (0%) |
| First incidence (days) | 731 (T) | — | 731 (T) | — |
| Poly-3 test | P=0.081N | P=0.116N | P=0.285N | P=0.108N |
| **Uterus: Stromal Polyp** | | | | |
| Overall rate | 3/50 (6%) | 0/50 (0%) | 2/50 (4%) | 2/50 (4%) |
| Adjusted rate | 6.8% | 0.0% | 4.3% | 4.2% |
| Terminal rate | 2/37 (5%) | 0/36 (0%) | 2/39 (5%) | 1/35 (3%) |
| First incidence (days) | 693 | — | 731 (T) | 677 |
| Poly-3 test | P=0.560N | P=0.116N | P=0.476N | P=0.471N |
| **All Organs: Hemangiosarcoma** | | | | |
| Overall rate | 1/50 (2%) | 3/50 (6%) | 6/50 (12%) | 1/50 (2%) |
| Adjusted rate | 2.3% | 6.6% | 12.8% | 2.1% |
| Terminal rate | 1/37 (3%) | 1/36 (3%) | 5/39 (13%) | 1/35 (3%) |
| First incidence (days) | 731 (T) | 555 | 673 | 731 (T) |
| Poly-3 test | P=0.518N | P=0.318 | P=0.066 | P=0.746N |
| **All Organs: Hemangioma or Hemangiosarcoma** | | | | |
| Overall rate | 1/50 (2%) | 3/50 (6%) | 6/50 (12%) | 3/50 (6%) |
| Adjusted rate | 2.3% | 6.6% | 12.8% | 6.4% |
| Terminal rate | 1/37 (3%) | 1/36 (3%) | 5/39 (13%) | 3/35 (9%) |
| First incidence (days) | 731 (T) | 555 | 673 | 731 (T) |
| Poly-3 test | P=0.304 | P=0.318 | P=0.066 | P=0.329 |
| **All Organs: Malignant Lymphoma** | | | | |
| Overall rate | 7/50 (14%) | 15/50 (30%) | 6/50 (12%) | 12/50 (24%) |
| Adjusted rate | 15.4% | 33.0% | 12.7% | 25.0% |
| Terminal rate | 5/37 (14%) | 12/36 (33%) | 4/39 (10%) | 7/35 (20%) |
| First incidence (days) | 292 | 645 | 639 | 565 |
| Poly-3 test | P=0.372 | P=0.041 | P=0.471N | P=0.187 |
| **All Organs: Benign Neoplasms** | | | | |
| Overall rate | 34/50 (68%) | 39/50 (78%) | 47/50 (94%) | 47/50 (94%) |
| Adjusted rate | 73.3% | 82.6% | 95.7% | 95.3% |
| Terminal rate | 29/37 (78%) | 30/36 (83%) | 38/39 (97%) | 34/35 (97%) |
| First incidence (days) | 193 | 555 | 495 | 565 |
| Poly-3 test | P<0.001 | P=0.192 | P<0.001 | P=0.002 |
| **All Organs: Malignant Neoplasms** | | | | |
| Overall rate | 26/50 (52%) | 33/50 (66%) | 37/50 (74%) | 43/50 (86%) |
| Adjusted rate | 55.3% | 67.6% | 74.0% | 86.0% |
| Terminal rate | 19/37 (51%) | 21/36 (58%) | 26/39 (67%) | 28/35 (80%) |
| First incidence (days) | 292 | 481 | 495 | 513 |
| Poly-3 test | P<0.001 | P=0.149 | P=0.040 | P<0.001 |

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

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **All Organs: Benign or Malignant Neoplasms** | | | | |
| Overall rate | 42/50 (84%) | 44/50 (88%) | 50/50 (100%) | 50/50 (100%) |
| Adjusted rate | 85.9% | 90.2% | 100% | 100% |
| Terminal rate | 32/37 (87%) | 32/36 (89%) | 39/39 (100%) | 35/35 (100%) |
| First incidence (days) | 193 | 481 | 495 | 513 |
| Poly-3 test | P<0.001 | P=0.366 | P=0.007 | P=0.007 |

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, pituitary gland, spleen, 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 are the P values 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] One animal with adenoma also had hepatoblastoma.
[g] Value of statistic cannot be computed.

**TABLE D3a**
**Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F1 Mice**

| Study | Incidence in Controls | | |
|---|---|---|---|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| **Historical Incidence: Inhalation Studies** | | | |
| Cumene | 1/50 | 3/50 | 4/50 |
| Decalin | 1/49 | 6/49 | 7/49 |
| Divinylbenzene | 4/50 | 2/50 | 6/50 |
| Methyl isobutyl ketone | 4/50 | 0/50 | 4/50 |
| α-Methylstyrene | 1/50 | 1/50 | 2/50 |
| Propargyl alcohol | 3/50 | 2/50 | 5/50 |
| Propylene glycol mono-*t*-butyl ether | 2/50 | 1/50 | 3/50 |
| Stoddard solvent IIC | 2/50 | 0/50 | 2/50 |
| Vanadium pentoxide | 1/50 | 0/50 | 1/50 |
| **Overall Historical Incidence: Inhalation Studies** | | | |
| Total (%) | 19/449 (4.2%) | 15/449 (3.3%) | 34/449 (7.6%) |
| Mean ± standard deviation | 4.2% ± 2.5% | 3.4% ± 3.9% | 7.6% ± 4.0% |
| Range | 2%-8% | 0%-12% | 2%-14% |
| **Overall Historical Incidence: All Routes** | | | |
| Total (%) | 77/1,596 (4.8%) | 57/1,596 (3.6%) | 129/1,596 (8.1%) |
| Mean ± standard deviation | 4.9% ± 2.7% | 3.6% ± 3.1% | 8.2% ± 3.9% |
| Range | 0%-12% | 0%-12% | 2%-18% |

[a] Data as of March 2, 2007

**TABLE D3b**
**Historical Incidence of Hepatocellular Neoplasms in Control Female B6C3F1 Mice[a]**

| Study | Incidence in Controls | | |
|---|---|---|---|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| **Historical Incidence: Inhalation Studies** | | | |
| Cumene | 18/50 | 10/50 | 25/50 |
| Decalin | 7/49 | 4/49 | 11/49 |
| Divinylbenzene | 17/49 | 5/49 | 19/49 |
| Methyl isobutyl ketone | 13/50 | 6/50 | 17/50 |
| α-Methylstyrene | 10/50 | 3/50 | 13/50 |
| Propargyl alcohol | 15/50 | 4/50 | 17/50 |
| Propylene glycol mono-*t*-butyl ether | 14/49 | 4/49 | 18/49 |
| Stoddard solvent IIC | 9/50 | 6/50 | 13/50 |
| Vanadium pentoxide | 6/50 | 6/50 | 12/50 |
| **Overall Historical Incidence: Inhalation Studies** | | | |
| Total (%) | 109/447 (24.4%) | 48/447 (10.7%) | 145/447 (32.4%) |
| Mean ± standard deviation | 24.4% ± 8.7% | 10.7% ± 4.1% | 32.4% ± 8.8% |
| Range | 12%-36% | 6%-20% | 22%-50% |
| **Overall Historical Incidence: All Routes** | | | |
| Total (%) | 402/1,593 (25.2%) | 159/1,593 (10.0%) | 505/1,593 (31.7%) |
| Mean ± standard deviation | 25.8% ± 15.8% | 10.2% ± 6.6% | 32.4% ± 17.5% |
| Range | 2%-62% | 0%-28% | 8%-64% |

[a] Data as of March 2, 2007

**TABLE D3c**
**Historical Incidence of Hemangiosarcoma in Control Female B6C3F1 Mice[a]**

| Study | Incidence in Controls | |
|---|---|---|
| | Spleen | All Organs |
| **Historical Incidence: Inhalation Studies** | | |
| Cumene | 0/49 | 1/50 |
| Decalin | 1/49 | 1/49 |
| Divinylbenzene | 0/49 | 1/50 |
| Methyl isobutyl ketone | 1/50 | 3/50 |
| α-Methylstyrene | 0/50 | 4/50 |
| Propargyl alcohol | 1/50 | 2/50 |
| Propylene glycol mono-*t*-butyl ether | 0/49 | 1/50 |
| Stoddard solvent IIC | 1/49 | 1/50 |
| Vanadium pentoxide | 2/50 | 2/50 |
| **Overall Historical Incidence: Inhalation Studies** | | |
| Total (%) | 6/445 (1.4%) | 16/449 (3.6%) |
| Mean ± standard deviation | 1.3% ± 1.4% | 3.6% ± 2.2% |
| Range | 0%-4% | 2%-8% |
| **Overall Historical Incidence: All Routes** | | |
| Total (%) | 27/1,573 (1.7%) | 71/1,598 (4.4%) |
| Mean ± standard deviation | 1.8% ± 1.8% | 4.6% ± 3.2% |
| Range | 0%-8% | 2%-16% |

[a] Data as of March 2, 2007

**TABLE D4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Cumene[a]**

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 8 | 10 | 8 | 12 |
| Natural deaths | 5 | 4 | 3 | 3 |
| Survivors | | | | |
| Died last week of study | | | | 1 |
| Terminal sacrifice | 37 | 36 | 39 | 34 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Esophagus | (50) | (50) | (50) | (50) |
| Gallbladder | (38) | (41) | (44) | (37) |
| Intestine large, cecum | (46) | (47) | (49) | (47) |
| Intestine large, colon | (47) | (50) | (50) | (48) |
| Intestine small, duodenum | (46) | (47) | (49) | (48) |
| Necrosis | 1 (2%) | | | |
| Intestine small, ileum | (46) | (47) | (49) | (47) |
| Intestine small, jejunum | (46) | (47) | (49) | (47) |
| Hyperplasia, lymphoid | | | 1 (2%) | |
| Liver | (50) | (50) | (50) | (50) |
| Angiectasis | | 1 (2%) | 1 (2%) | |
| Basophilic focus | 3 (6%) | 3 (6%) | 4 (8%) | 1 (2%) |
| Clear cell focus | 4 (8%) | 6 (12%) | 1 (2%) | 5 (10%) |
| Cyst | 1 (2%) | | | 1 (2%) |
| Eosinophilic focus | 8 (16%) | 11 (22%) | 7 (14%) | 14 (28%) |
| Erythrophagocytosis | | | | 1 (2%) |
| Fatty change | 1 (2%) | | | |
| Hematopoietic cell proliferation | | 1 (2%) | | |
| Inflammation, granulomatous | 1 (2%) | | | |
| Mixed cell focus | | 1 (2%) | | 1 (2%) |
| Necrosis | 4 (8%) | 3 (6%) | 3 (6%) | 2 (4%) |
| Tension lipidosis | 3 (6%) | 3 (6%) | 4 (8%) | 4 (8%) |
| Centrilobular, necrosis | 2 (4%) | 1 (2%) | | 1 (2%) |
| Mesentery | (11) | (12) | (11) | (9) |
| Inflammation, chronic active | | | 1 (9%) | |
| Fat, hemorrhage | | 1 (8%) | | |
| Fat, necrosis | 11 (100%) | 10 (83%) | 10 (91%) | 9 (100%) |
| Pancreas | (49) | (50) | (50) | (50) |
| Atrophy | | | 1 (2%) | 3 (6%) |
| Inflammation, chronic active | 1 (2%) | | | |
| Salivary glands | (50) | (50) | (50) | (50) |
| Stomach, forestomach | (49) | (50) | (50) | (50) |
| Hemorrhage | | | 1 (2%) | |
| Inflammation | 1 (2%) | | | |
| Ulcer | | | 1 (2%) | 2 (4%) |
| Epithelium, erosion | | | | 1 (2%) |
| Epithelium, hyperplasia | 1 (2%) | 2 (4%) | 2 (4%) | 3 (6%) |
| Stomach, glandular | (49) | (50) | (50) | (49) |
| Mineralization | | | 1 (2%) | |
| Necrosis | 1 (2%) | | | 1 (2%) |

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

**TABLE D4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Cardiovascular System** | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Cardiomyopathy | 12 (24%) | 17 (34%) | 6 (12%) | 7 (14%) |
| Inflammation, chronic | | | 1 (2%) | |
| Mineralization | | 1 (2%) | 1 (2%) | |
| Thrombosis | | 1 (2%) | | |
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (50) | (49) |
| Hyperplasia | 5 (10%) | 7 (14%) | 7 (14%) | 5 (10%) |
| Hypertrophy | 2 (4%) | 1 (2%) | 1 (2%) | 6 (12%) |
| Adrenal medulla | (50) | (49) | (50) | (49) |
| Hyperplasia | 1 (2%) | 4 (8%) | 2 (4%) | 2 (4%) |
| Islets, pancreatic | (49) | (50) | (50) | (50) |
| Hyperplasia | 1 (2%) | 1 (2%) | 2 (4%) | 3 (6%) |
| Pituitary gland | (50) | (49) | (50) | (48) |
| Pars distalis, angiectasis | 3 (6%) | 1 (2%) | 7 (14%) | 1 (2%) |
| Pars distalis, hyperplasia | 11 (22%) | 19 (39%) | 10 (20%) | 6 (13%) |
| Thyroid gland | (50) | (50) | (50) | (50) |
| Follicular cell, hyperplasia | 15 (30%) | 10 (20%) | 16 (32%) | 10 (20%) |
| **General Body System** | | | | |
| None | | | | |
| **Genital System** | | | | |
| Ovary | (48) | (50) | (50) | (49) |
| Angiectasis | 1 (2%) | | | |
| Cyst | 9 (19%) | 12 (24%) | 14 (28%) | 11 (22%) |
| Thrombosis | 1 (2%) | 1 (2%) | | 1 (2%) |
| Uterus | (50) | (50) | (50) | (50) |
| Angiectasis | 1 (2%) | 2 (4%) | 1 (2%) | 3 (6%) |
| Decidual reaction | 1 (2%) | | | |
| Inflammation, suppurative | 1 (2%) | 2 (4%) | | 1 (2%) |
| Thrombosis | 1 (2%) | | | 2 (4%) |
| Endometrium, hyperplasia, cystic | 8 (16%) | 11 (22%) | 18 (36%) | 8 (16%) |
| **Hematopoietic System** | | | | |
| Bone marrow | (49) | (50) | (50) | (50) |
| Lymph node | (4) | (9) | (5) | (5) |
| Iliac, ectasia | 1 (25%) | 1 (11%) | | |
| Lumbar, angiectasis | | 1 (11%) | | |
| Lumbar, ectasia | 2 (50%) | 1 (11%) | 1 (20%) | 1 (20%) |
| Lumbar, hyperplasia, lymphoid | | 2 (22%) | | |
| Renal, ectasia | 1 (25%) | | | |
| Lymph node, bronchial | (39) | (36) | (40) | (46) |
| Lymph node, mandibular | (35) | (42) | (38) | (42) |
| Inflammation, granulomatous | 1 (3%) | | | |
| Lymph node, mediastinal | (44) | (40) | (42) | (40) |
| Hyperplasia, lymphoid | | | | 1 (3%) |
| Lymph node, mesenteric | (46) | (46) | (48) | (48) |

**TABLE D4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Hematopoietic System** (continued) | | | | |
| Spleen | (49) | (50) | (50) | (50) |
| Hematopoietic cell proliferation | 1 (2%) | 6 (12%) | 2 (4%) | 2 (4%) |
| Hyperplasia, lymphoid | 1 (2%) | | | 1 (2%) |
| Thymus | (49) | (50) | (48) | (48) |
| **Integumentary System** | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Hyperplasia | 1 (2%) | | | 1 (2%) |
| Skin | (50) | (50) | (50) | (50) |
| Inflammation, chronic active | | 1 (2%) | 1 (2%) | |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Skeletal muscle | (1) | (1) | (1) | |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Necrosis | | | 1 (2%) | |
| Meninges, infiltration cellular, mononuclear cell | | | | 1 (2%) |
| **Respiratory System** | | | | |
| Larynx | (50) | (49) | (50) | (50) |
| Inflammation, suppurative | | | | 1 (2%) |
| Metaplasia, squamous | 1 (2%) | | | |
| Lung | (50) | (50) | (50) | (50) |
| Foreign body | | | | 1 (2%) |
| Hemorrhage | | 2 (4%) | | |
| Inflammation, suppurative | | | | 1 (2%) |
| Thrombosis | | 2 (4%) | | |
| Alveolar epithelium, bronchiole, hyperplasia | | | 1 (2%) | |
| Alveolar epithelium, bronchiole, metaplasia | | 42 (84%) | 49 (98%) | 47 (94%) |
| Alveolar epithelium, hyperplasia | 2 (4%) | 3 (6%) | 6 (12%) | 6 (12%) |
| Alveolar epithelium, metaplasia, squamous | 1 (2%) | | | |
| Alveolus, infiltration cellular, histiocyte | | 1 (2%) | 1 (2%) | 3 (6%) |
| Bronchiole, hyperplasia | | 17 (34%) | 10 (20%) | 14 (28%) |
| Nose | (50) | (50) | (50) | (50) |
| Inflammation, suppurative | | 1 (2%) | 3 (6%) | 7 (14%) |
| Glands, olfactory epithelium, cyst | | | 1 (2%) | |
| Glands, olfactory epithelium, hyperplasia | 1 (2%) | 4 (8%) | 4 (8%) | 11 (22%) |
| Olfactory epithelium, atrophy | 4 (8%) | 11 (22%) | 9 (18%) | 18 (36%) |
| Olfactory epithelium, hyperplasia, atypical | | | 2 (4%) | 10 (20%) |
| Olfactory epithelium, hyperplasia, basal cell | | 1 (2%) | 11 (22%) | 25 (50%) |
| Olfactory epithelium, necrosis | | 2 (4%) | 2 (4%) | 3 (6%) |
| Respiratory epithelium, accumulation, hyaline droplet | | 1 (2%) | 1 (2%) | |
| Respiratory epithelium, metaplasia, squamous | | | 1 (2%) | 6 (12%) |
| Respiratory epithelium, necrosis | | | 1 (2%) | |
| Turbinate, necrosis | | | | 2 (4%) |
| Pleura | | (1) | (1) | (2) |
| Trachea | (50) | (50) | (50) | (50) |
| Inflammation, suppurative | | | | 2 (4%) |

**TABLE D4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Cumene**

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| **Special Senses System** | | | | |
| Eye | (49) | (50) | (50) | (49) |
| Cataract | 2 (4%) | | 1 (2%) | 2 (4%) |
| Degeneration | | | | 1 (2%) |
| Cornea, epithelium, hyperplasia | | | | 1 (2%) |
| Cornea, inflammation, acute | 1 (2%) | | 1 (2%) | |
| Cornea, inflammation, chronic active | 4 (8%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Cornea, mineralization | | | | 1 (2%) |
| Harderian gland | (50) | (49) | (50) | (50) |
| Atrophy | 1 (2%) | | | |
| Hyperplasia | 6 (12%) | 2 (4%) | 1 (2%) | 1 (2%) |
| **Urinary System** | | | | |
| Kidney | (49) | (50) | (50) | (50) |
| Cyst | | 1 (2%) | | |
| Infarct | 1 (2%) | 3 (6%) | 2 (4%) | 1 (2%) |
| Inflammation, suppurative | | | | 1 (2%) |
| Metaplasia, osseous | 2 (4%) | 3 (6%) | 1 (2%) | |
| Nephropathy | 36 (73%) | 34 (68%) | 34 (68%) | 34 (68%) |
| Capsule, fibrosis | 1 (2%) | | 1 (2%) | |
| Renal tubule, karyomegaly | 1 (2%) | | | |
| Renal tubule, necrosis | 3 (6%) | 1 (2%) | | 1 (2%) |
| Urinary bladder | (48) | (48) | (49) | (48) |
| Inflammation, chronic active | | | 1 (2%) | |
| Artery, inflammation, chronic active | | 1 (2%) | | |

# APPENDIX E
# GENETIC TOXICOLOGY

# GENETIC TOXICOLOGY

## *SALMONELLA TYPHIMURIUM* MUTAGENICITY TEST PROTOCOL

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

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of cumene. The high dose was limited by toxicity. All trials were repeated; negative trials conducted with S9 were repeated with a higher S9 concentration.

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

## RAT BONE MARROW MICRONUCLEUS TEST PROTOCOL

Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by cumene exposure. A high dose of 2,500 mg/kg was selected based on toxicity. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male F344/N rats were injected intraperitoneally (three times at 24-hour intervals) with cumene dissolved in corn oil. Vehicle control animals were injected with corn oil only. The positive control animals received injections of cyclophosphamide (25 mg/kg). The animals were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in up to five rats per dose group. In addition, the percentage of PCEs among the total erythrocyte population in the bone marrow was scored for each dose group as a measure of cumene-induced bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted previously). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

## MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 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 micronucleated cells in 1,000 normochromatic erythrocytes (NCEs) in each of 9 or 10 mice per exposure group. In addition, the percentage of PCEs in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as described for PCEs in the rat bone marrow micronucleus test. Results of the 3-month study were accepted without repeat tests because additional data could not be obtained.

## EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The 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

Cumene (1 to 333 µg/plate) was not mutagenic in *S. typhimurium* strain TA97, TA98, TA100, or TA1535 when tested with and without induced rat or hamster liver S9 activation enzymes (Table E1). *In vivo*, cumene induced small, but significant, increases in micronucleated PCEs in bone marrow of male rats treated by intraperitoneal injection (Table E2). Two trials were performed in rats. In the first trial, doses ranging from 78.13 to 2,500 mg/kg were administered three times at 24-hour intervals, and results were positive, based both on the trend ($P<0.001$) and the response at the 1,250 mg/kg dose. The data from the 2,500 mg/kg dose were excluded from analysis because only two animals survived and a minimum of three animals is required for a valid dose point. The second confirmatory trial also produced a positive response, although the trend test was not significant ($P=0.085$). Micronucleated erythrocytes were elevated at all four doses in trial 2; the responses at the 312 and 1,250 mg/kg levels were statistically significant ($P<0.006$). Because considerable toxicity was again observed at the high dose of 2,500 mg/kg, the trend was recalculated over the same dose range as for the first trial (0 to 1,250 mg/kg), and a significant P value of 0.019 was produced. The percentage of PCEs in the bone marrow fluctuated unrelated to dose and likely represented variation within the normal range of 40% to 60% PCEs among the total erythrocyte population in the bone marrow. In contrast to the results in male rats, no increase in micronucleated erythrocytes was observed in peripheral blood of male or female mice exposed to cumene by inhalation (62.5 to 1,000 ppm) for 3 months (Table E3). For both male and female mice, no significant changes in the percentage of PCEs were observed over the exposure range tested, indicating an absence of treatment-related toxicity to the bone marrow.

**TABLE E1**
**Mutagenicity of Cumene in *Salmonella typhimurium*[a]**

| Strain | Dose (µg/plate) | Revertants/Plate[b] –S9 Trial 1 | –S9 Trial 2 | +hamster S9 10% | +hamster S9 30% | +rat S9 10% | +rat S9 30% |
|---|---|---|---|---|---|---|---|
| **TA100** | 0 | 99 ± 5.0 | 105 ± 1.0 | 121 ± 11.0 | 126 ± 20.0 | 125 ± 7.0 | 125 ± 11.0 |
| | 1 | | 100 ± 8.0 | | | | |
| | 3 | 103 ± 3.0 | 104 ± 4.0 | 107 ± 4.0 | 119 ± 6.0 | 112 ± 8.0 | 137 ± 11.0 |
| | 10 | 93 ± 6.0 | 116 ± 7.0 | 112 ± 8.0 | 127 ± 8.0 | 116 ± 10.0 | 129 ± 3.0 |
| | 33 | 96 ± 5.0 | 93 ± 6.0 | 102 ± 7.0 | 105 ± 7.0 | 113 ± 5.0 | 122 ± 4.0 |
| | 100 | 100 ± 1.0 | 111 ± 8.0 | 102 ± 3.0 | 112 ± 7.0 | 105 ± 3.0 | 138 ± 5.0 |
| | 166 | 47 ± 14.0[c] | | 106 ± 6.0 | | 95 ± 5.0 | |
| | 333 | | | | 79 ± 4.0[c] | | 91 ± 9.0 |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control[d] | | 914 ± 8.0 | 875 ± 26.0 | 1,420 ± 121.0 | 603 ± 25.0 | 1,111 ± 30.0 | 792 ± 16.0 |
| **TA1535** | 0 | 15 ± 1.0 | 14 ± 0.0 | 15 ± 2.0 | 14 ± 1.0 | 21 ± 2.0 | 13 ± 3.0 |
| | 1 | 10 ± 2.0 | 13 ± 2.0 | | | | |
| | 3 | 17 ± 0.0 | 13 ± 1.0 | 12 ± 2.0 | 12 ± 1.0 | 17 ± 2.0 | 14 ± 0.0 |
| | 10 | 14 ± 1.0 | 13 ± 3.0 | 17 ± 1.0 | 9 ± 2.0 | 17 ± 1.0 | 14 ± 2.0 |
| | 33 | 13 ± 1.0 | 12 ± 1.0 | 11 ± 1.0 | 14 ± 3.0 | 15 ± 2.0 | 12 ± 2.0 |
| | 100 | 11 ± 2.0 | 16 ± 5.0 | 10 ± 2.0 | 15 ± 1.0 | 12 ± 2.0 | 12 ± 2.0 |
| | 166 | | | 9 ± 1.0 | 10 ± 2.0 | 11 ± 1.0 | 11 ± 4.0 |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 851 ± 8.0 | 399 ± 17.0 | 198 ± 12.0 | 321 ± 15.0 | 140 ± 2.0 | 222 ± 7.0 |
| **TA97** | 0 | 133 ± 12.0 | 156 ± 7.0 | 188 ± 2.0 | 159 ± 1.0 | 192 ± 4.0 | 170 ± 11.0 |
| | 1 | 128 ± 1.0 | 177 ± 3.0 | | | | |
| | 3 | 144 ± 5.0 | 174 ± 2.0 | 181 ± 6.0 | 165 ± 6.0 | 172 ± 8.0 | 189 ± 5.0 |
| | 10 | 148 ± 12.0 | 172 ± 2.0 | 185 ± 3.0 | 171 ± 4.0 | 176 ± 7.0 | 191 ± 3.0 |
| | 33 | 136 ± 13.0 | 158 ± 4.0 | 195 ± 9.0 | 185 ± 4.0 | 190 ± 4.0 | 188 ± 2.0 |
| | 100 | 99 ± 8.0 | 53 ± 17.0[c] | 153 ± 1.0 | 186 ± 7.0 | 149 ± 15.0 | 168 ± 15.0 |
| | 166 | | | 125 ± 9.0[c] | 153 ± 4.0 | 115 ± 9.0[c] | 163 ± 2.0 |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 426 ± 13.0 | 515 ± 13.0 | 545 ± 22.0 | 481 ± 18.0 | 478 ± 12.0 | 435 ± 23.0 |
| **TA98** | 0 | 9 ± 1.0 | 20 ± 1.0 | 22 ± 3.0 | 14 ± 3.0 | 19 ± 1.0 | 19 ± 1.0 |
| | 1 | | 22 ± 4.0 | | | | |
| | 3 | 9 ± 0.0 | 16 ± 3.0 | 22 ± 2.0 | 14 ± 2.0 | 25 ± 5.0 | 11 ± 2.0 |
| | 10 | 9 ± 1.0 | 20 ± 2.0 | 23 ± 1.0 | 13 ± 3.0 | 24 ± 3.0 | 14 ± 3.0 |
| | 33 | 14 ± 1.0 | 18 ± 5.0 | 22 ± 3.0 | 13 ± 3.0 | 16 ± 1.0 | 13 ± 1.0 |
| | 100 | 8 ± 0.0 | 16 ± 2.0 | 23 ± 2.0 | 18 ± 2.0 | 14 ± 1.0 | 11 ± 2.0 |
| | 166 | 4 ± 0.0[c] | | 21 ± 3.0 | | 23 ± 1.0 | |
| | 333 | | | | 10 ± 1.0[c] | | 12 ± 3.0 |
| Trial summary | | Negative | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 328 ± 28.0 | 414 ± 24.0 | 1,227 ± 35.0 | 291 ± 13.0 | 890 ± 63.0 | 669 ± 17.0 |

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

**TABLE E2**
**Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with Cumene by Intraperitoneal Injection[a]**

| Compound | Dose (mg/kg) | Number of Male Rats with Erythrocytes Scored | Micronucleated PCEs/ 1,000 PCEs[b] | Pairwise P Value[c] | PCE[b] (%) |
|---|---|---|---|---|---|
| **Trial 1** | | | | | |
| Corn oil[d] | 0 | 5 | 0.50 ± 0.16 | | 50.2 ± 2.9 |
| Cumene | 78.13 | 5 | 1.20 ± 0.25 | 0.0447 | 59.4 ± 5.1 |
| | 156.25 | 5 | 1.20 ± 0.34 | 0.0447 | 64.8 ± 4.2 |
| | 312.5 | 5 | 1.30 ± 0.54 | 0.0296 | 54.6 ± 3.1 |
| | 625 | 5 | 0.80 ± 0.41 | 0.2026 | 45.1 ± 1.7 |
| | 1,250 | 5 | 2.60 ± 0.29 | 0.0001 | 46.6 ± 4.8 |
| | 2,500 | 2[e] | 1.25 ± 0.25 | | 49.3 ± 2.8 |
| | | | P<0.001[f] | | |
| Cyclophosphamide[g] | 25 | 5 | 17.30 ± 2.32 | 0.0000 | 50.3 ± 4.3 |
| **Trial 2** | | | | | |
| Corn oil | 0 | 5 | 0.50 ± 0.27 | | 53.2 ± 3.8 |
| Cumene | 312 | 5 | 1.70 ± 0.20 | 0.0052 | 50.2 ± 1.0 |
| | 625 | 5 | 1.40 ± 0.33 | 0.0194 | 47.6 ± 3.1 |
| | 1,250 | 5 | 1.80 ± 0.34 | 0.0033 | 44.5 ± 3.0 |
| | 2,500 | 3 | 1.50 ± 1.00 | 0.0192 | 54.3 ± 2.1 |
| | | | P=0.085 | | |
| Cyclophosphamide | 25 | 5 | 7.80 ± 1.63 | 0.0000 | 38.7 ± 2.7 |

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Shelby *et al.* (1993).
[b] PCE=polychromatic erythrocyte
[c] Mean ± standard error
[d] Pairwise comparison with the vehicle control; dosed group values are significant at P≤0.005 (trial 1) or P≤0.006 (trial 2); positive control values are significant at P≤0.05 (ILS, 1990)
[e] Vehicle control
[f] Statistical tests not performed due to high mortality
[g] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990); 2,500 mg/kg group excluded due to high mortality
Positive control

**TABLE E3**
**Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Cumene by Inhalation for 3 Months[a]**

| Compound | Concentration (ppm) | Number of Mice with Erythrocytes Scored | Micronucleated NCEs/ 1,000 NCEs[b] | P-Value[c] | PCEs[b] (%) |
|---|---|---|---|---|---|
| **Male** | | | | | |
| Air[d] | 0 | 10 | 2.40 ± 0.69 | | 2.7 ± 0.1 |
| Cumene | 62.5 | 10 | 2.20 ± 0.66 | 0.6161 | 2.6 ± 0.1 |
| | 125 | 10 | 2.10 ± 0.48 | 0.6728 | 2.6 ± 0.1 |
| | 250 | 10 | 1.80 ± 0.36 | 0.8230 | 2.8 ± 0.1 |
| | 500 | 10 | 2.00 ± 0.26 | 0.7270 | 2.9 ± 0.1 |
| | 1,000 | 10 | 2.20 ± 0.42 | 0.6161 | 2.9 ± 0.2 |
| | | | P=0.553[e] | | |
| **Female** | | | | | |
| Air | 0 | 10 | 2.30 ± 0.40 | | 3.3 ± 0.1 |
| Cumene | 62.5 | 9 | 1.33 ± 0.37 | 0.9396 | 2.3 ± 0.1 |
| | 125 | 10 | 1.70 ± 0.30 | 0.8289 | 3.1 ± 0.2 |
| | 250 | 10 | 2.10 ± 0.53 | 0.6186 | 3.3 ± 0.2 |
| | 500 | 10 | 2.10 ± 0.35 | 0.6186 | 3.4 ± 0.1 |
| | | | P=0.329 | | |

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al*. (1990).
[b] NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[c] Mean ± standard error
[d] Pairwise comparison with the chamber controls, significant at P≤0.005 (males) or P≤0.006 (females) (ILS, 1990)
[e] Chamber control
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 and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene[a]**

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| Hematology | | | | | | |
| n | | | | | | |
| Day 3 | 10 | 10 | 10 | 10 | 10 | 10 |
| Day 23 | 10 | 10 | 10 | 10 | 10 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 10 | 9 |
| Hematocrit (%) | | | | | | |
| Day 3 | 43.6 ± 0.7 | 42.1 ± 0.4 | 42.7 ± 0.4 | 42.9 ± 0.5 | 42.9 ± 0.5 | 42.5 ± 0.4 |
| Day 23 | 49.0 ± 0.5 | 48.4 ± 0.4 | 48.1 ± 0.5 | 47.7 ± 0.4 | 48.4 ± 0.3 | 48.9 ± 0.5 |
| Week 14 | 45.5 ± 0.4 | 45.4 ± 0.3 | 45.1 ± 0.4 | 44.3 ± 0.3 | 44.3 ± 0.3 | 44.4 ± 0.2 |
| Packed cell volume (mL/dL) | | | | | | |
| Day 3 | 41.3 ± 0.6 | 40.1 ± 0.5 | 40.5 ± 0.5 | 40.8 ± 0.5 | 40.7 ± 0.5 | 39.9 ± 0.4 |
| Day 23 | 48.0 ± 0.7 | 47.9 ± 0.5 | 47.2 ± 0.5 | 46.8 ± 0.5 | 46.9 ± 0.4 | 48.8 ± 0.5 |
| Week 14 | 45.5 ± 0.3 | 45.4 ± 0.5 | 44.8 ± 0.4 | 44.4 ± 0.5 | 44.1 ± 0.4 | 44.7 ± 0.3 |
| Hemoglobin (g/dL) | | | | | | |
| Day 3 | 13.4 ± 0.2 | 13.1 ± 0.2 | 12.8 ± 0.2 | 13.0 ± 0.1 | 12.9 ± 0.1 | 12.9 ± 0.1 |
| Day 23 | 15.2 ± 0.2 | 15.1 ± 0.1 | 15.0 ± 0.2 | 14.9 ± 0.1 | 15.1 ± 0.1 | 15.1 ± 0.2 |
| Week 14 | 15.1 ± 0.1 | 14.9 ± 0.1 | 14.9 ± 0.1 | 14.8 ± 0.1 | 14.7 ± 0.1* | 14.8 ± 0.1 |
| Erythrocytes ($10^6$/μL) | | | | | | |
| Day 3 | 6.47 ± 0.09 | 6.29 ± 0.09 | 6.23 ± 0.09 | 6.28 ± 0.09 | 6.33 ± 0.09 | 6.32 ± 0.09 |
| Day 23 | 7.87 ± 0.13 | 7.68 ± 0.10 | 7.56 ± 0.10 | 7.48 ± 0.10 | 7.53 ± 0.08 | 7.67 ± 0.10 |
| Week 14 | 8.20 ± 0.05 | 8.19 ± 0.08 | 8.11 ± 0.06 | 7.98 ± 0.09* | 7.95 ± 0.06* | 8.05 ± 0.06* |
| Reticulocytes ($10^6$/μL) | | | | | | |
| Day 3 | 0.25 ± 0.04 | 0.32 ± 0.03 | 0.35 ± 0.02* | 0.41 ± 0.03** | 0.43 ± 0.04** | 0.42 ± 0.04** |
| Day 23 | 0.20 ± 0.02 | 0.24 ± 0.03 | 0.30 ± 0.02* | 0.27 ± 0.02 | 0.23 ± 0.03 | 0.26 ± 0.02 |
| Week 14 | 0.18 ± 0.02 | 0.18 ± 0.01 | 0.19 ± 0.01 | 0.19 ± 0.01 | 0.19 ± 0.02 | 0.18 ± 0.02 |
| Nucleated erythrocytes/100 leukocytes | | | | | | |
| Day 3 | 1.00 ± 0.33 | 1.40 ± 0.40 | 2.20 ± 0.39* | 2.50 ± 0.54* | 2.30 ± 0.37* | 2.80 ± 0.33** |
| Day 23 | 0.10 ± 0.10 | 0.20 ± 0.13 | 0.20 ± 0.13 | 0.30 ± 0.21 | 0.10 ± 0.10 | 0.80 ± 0.29* |
| Week 14 | 0.30 ± 0.15 | 0.30 ± 0.15 | 0.50 ± 0.17 | 0.30 ± 0.15 | 0.10 ± 0.10 | 0.44 ± 0.34 |
| Mean cell volume (fL) | | | | | | |
| Day 3 | 64.0 ± 0.4 | 63.8 ± 0.3 | 64.7 ± 0.3 | 64.9 ± 0.4 | 64.4 ± 0.5 | 63.1 ± 0.7 |
| Day 23 | 61.0 ± 0.5 | 62.5 ± 0.3 | 62.7 ± 0.4 | 62.6 ± 0.4 | 62.3 ± 0.4 | 63.7 ± 0.4** |
| Week 14 | 55.6 ± 0.2 | 55.3 ± 0.2 | 55.4 ± 0.2 | 55.4 ± 0.2 | 55.5 ± 0.2 | 55.6 ± 0.2 |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 3 | 20.7 ± 0.2 | 20.9 ± 0.2 | 20.5 ± 0.2 | 20.7 ± 0.2 | 20.4 ± 0.3 | 20.5 ± 0.3 |
| Day 23 | 19.3 ± 0.2 | 19.7 ± 0.1 | 19.9 ± 0.2 | 19.9 ± 0.2 | 20.0 ± 0.2 | 19.7 ± 0.1 |
| Week 14 | 18.5 ± 0.1 | 18.3 ± 0.1 | 18.4 ± 0.1 | 18.5 ± 0.1 | 18.5 ± 0.1 | 18.5 ± 0.2 |
| Mean cell hemoglobin concentration (g/dL) | | | | | | |
| Day 3 | 32.5 ± 0.4 | 32.8 ± 0.2 | 31.6 ± 0.3 | 31.9 ± 0.4 | 31.7 ± 0.4 | 32.4 ± 0.2 |
| Day 23 | 31.7 ± 0.2 | 31.5 ± 0.1 | 31.8 ± 0.2 | 31.9 ± 0.2 | 32.1 ± 0.2 | 30.9 ± 0.2 |
| Week 14 | 33.3 ± 0.2 | 33.0 ± 0.2 | 33.3 ± 0.2 | 33.3 ± 0.2 | 33.4 ± 0.3 | 33.2 ± 0.2 |
| Platelets ($10^3$/μL) | | | | | | |
| Day 3 | 868.3 ± 23.8 | 859.6 ± 15.7 | 941.7 ± 28.7 | 924.3 ± 34.4 | 951.3 ± 24.1* | 958.2 ± 32.1* |
| Day 23 | 857.7 ± 19.8 | 858.7 ± 22.0 | 924.7 ± 30.8 | 880.3 ± 32.6 | 996.8 ± 34.9** | 1,075.7 ± 40.9** |
| Week 14 | 671.3 ± 8.4 | 677.6 ± 13.9 | 644.8 ± 10.1 | 679.0 ± 14.1 | 676.7 ± 7.6 | 757.6 ± 10.5** |

**TABLE F1**
**Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene**

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| **Male** (continued) | | | | | | |
| Hematology (continued) | | | | | | |
| n | | | | | | |
| Day 3 | 10 | 10 | 10 | 10 | 10 | 10 |
| Day 23 | 10 | 10 | 10 | 10 | 10 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 10 | 9 |
| Leukocytes ($10^3$/µL) | | | | | | |
| Day 3 | 7.74 ± 0.35 | 8.07 ± 0.24 | 8.24 ± 0.26 | 7.95 ± 0.30 | 8.00 ± 0.33 | 5.68 ± 0.58 |
| Day 23 | 7.64 ± 0.44 | 8.98 ± 0.36 | 8.30 ± 0.38 | 8.88 ± 0.37* | 9.68 ± 0.28** | 9.76 ± 0.30** |
| Week 14 | 5.54 ± 0.45 | 5.35 ± 0.40 | 6.07 ± 0.60 | 6.21 ± 0.41 | 7.91 ± 0.39** | 6.13 ± 0.58* |
| Segmented neutrophils ($10^3$/µL) | | | | | | |
| Day 3 | 0.80 ± 0.07 | 0.87 ± 0.10 | 1.02 ± 0.08 | 1.00 ± 0.12 | 1.00 ± 0.05 | 0.72 ± 0.09 |
| Day 23 | 0.78 ± 0.12 | 0.71 ± 0.08 | 0.90 ± 0.12 | 1.02 ± 0.08 | 1.29 ± 0.44 | 0.98 ± 0.08 |
| Week 14 | 0.95 ± 0.08 | 0.87 ± 0.14 | 1.05 ± 0.09 | 0.93 ± 0.09 | 1.12 ± 0.14 | 0.97 ± 0.09 |
| Bands ($10^3$/µL) | | | | | | |
| Day 3 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.02 ± 0.01 | 0.01 ± 0.01 | 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 | 0.01 ± 0.01 | 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 | 6.68 ± 0.34 | 6.93 ± 0.22 | 6.96 ± 0.23 | 6.69 ± 0.23 | 6.85 ± 0.33 | 4.82 ± 0.49* |
| Day 23 | 6.64 ± 0.45 | 8.08 ± 0.34 | 7.28 ± 0.29 | 7.67 ± 0.29 | 8.18 ± 0.39** | 8.50 ± 0.31** |
| Week 14 | 4.48 ± 0.40 | 4.35 ± 0.27 | 4.88 ± 0.52 | 5.13 ± 0.36 | 6.67 ± 0.38** | 5.07 ± 0.55* |
| Monocytes ($10^3$/µL) | | | | | | |
| Day 3 | 0.22 ± 0.05 | 0.21 ± 0.04 | 0.19 ± 0.04 | 0.21 ± 0.04 | 0.12 ± 0.03 | 0.09 ± 0.02* |
| Day 23 | 0.21 ± 0.06 | 0.19 ± 0.05 | 0.10 ± 0.05 | 0.17 ± 0.06 | 0.13 ± 0.05 | 0.22 ± 0.05 |
| Week 14 | 0.07 ± 0.01 | 0.11 ± 0.02 | 0.09 ± 0.02 | 0.05 ± 0.02 | 0.10 ± 0.02 | 0.05 ± 0.01 |
| Basophils ($10^3$/µL) | | | | | | |
| Day 3 | 0.015 ± 0.015 | 0.007 ± 0.007 | 0.025 ± 0.013 | 0.006 ± 0.006 | 0.000 ± 0.000 | 0.005 ± 0.005 |
| Day 23 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.011 ± 0.011 |
| 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 | 0.02 ± 0.01 | 0.07 ± 0.03 | 0.03 ± 0.02 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.05 ± 0.01 |
| Day 23 | 0.03 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.04 ± 0.02 | 0.07 ± 0.03 | 0.05 ± 0.02 |
| Week 14 | 0.04 ± 0.03 | 0.03 ± 0.01 | 0.05 ± 0.02 | 0.11 ± 0.03* | 0.03 ± 0.01 | 0.04 ± 0.02 |
| Clinical Chemistry | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 3 | 9.3 ± 0.8 | 7.5 ± 0.3 | 7.2 ± 0.2 | 6.7 ± 0.4 | 8.4 ± 0.4 | 12.5 ± 0.6 |
| Day 23 | 10.0 ± 0.7 | 10.2 ± 0.5 | 9.4 ± 0.3 | 8.6 ± 0.6 | 10.1 ± 0.3 | 13.6 ± 0.7* |
| Week 14 | 13.9 ± 0.4 | 13.5 ± 0.4 | 14.7 ± 0.3 | 14.0 ± 0.3 | 14.3 ± 0.4 | 14.2 ± 0.3 |
| Creatinine (mg/dL) | | | | | | |
| Day 3 | 0.71 ± 0.02 | 0.73 ± 0.02 | 0.70 ± 0.02 | 0.71 ± 0.01 | 0.72 ± 0.01 | 0.69 ± 0.01 |
| Day 23 | 0.72 ± 0.01 | 0.74 ± 0.02 | 0.73 ± 0.02 | 0.71 ± 0.01 | 0.71 ± 0.01 | 0.74 ± 0.02 |
| Week 14 | 1.05 ± 0.03 | 1.08 ± 0.04 | 1.05 ± 0.02 | 1.11 ± 0.03 | 1.11 ± 0.03 | 1.06 ± 0.03 |
| Total protein (g/dL) | | | | | | |
| Day 3 | 5.6 ± 0.1 | 5.5 ± 0.1 | 5.4 ± 0.1 | 5.3 ± 0.1 | 5.4 ± 0.1 | 5.4 ± 0.1 |
| Day 23 | 6.0 ± 0.0 | 6.1 ± 0.1 | 6.1 ± 0.0 | 6.1 ± 0.0 | 6.2 ± 0.1 | 6.4 ± 0.1** |
| Week 14 | 6.7 ± 0.1 | 6.8 ± 0.1 | 6.7 ± 0.0 | 6.8 ± 0.1 | 6.8 ± 0.2 | 7.0 ± 0.0** |

**TABLE F1**
**Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene**

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| **Male** (continued) | | | | | | |
| Clinical Chemistry (continued) | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Albumin (g/dL) | | | | | | |
| Day 3 | 4.0 ± 0.1 | 3.8 ± 0.1 | 3.6 ± 0.1* | 3.6 ± 0.1** | 3.8 ± 0.1 | 3.8 ± 0.1 |
| Day 23 | 3.8 ± 0.1 | 3.7 ± 0.1 | 3.8 ± 0.1 | 3.8 ± 0.1 | 3.9 ± 0.1 | 3.9 ± 0.0 |
| Week 14 | 3.8 ± 0.1 | 3.9 ± 0.0 | 3.8 ± 0.0 | 3.9 ± 0.0 | 4.1 ± 0.1** | 4.2 ± 0.0** |
| Globulin (g/dL) | | | | | | |
| Day 3 | 1.6 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.0 | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.6 ± 0.1 |
| Day 23 | 2.2 ± 0.1 | 2.4 ± 0.1 | 2.3 ± 0.0 | 2.3 ± 0.1 | 2.3 ± 0.1 | 2.4 ± 0.1 |
| Week 14 | 2.9 ± 0.0 | 2.8 ± 0.1 | 2.9 ± 0.0 | 3.0 ± 0.1 | 2.7 ± 0.2 | 2.9 ± 0.0 |
| Albumin/globulin ratio | | | | | | |
| Day 3 | 2.5 ± 0.2 | 2.4 ± 0.1 | 2.1 ± 0.1 | 2.1 ± 0.1 | 2.3 ± 0.1 | 2.5 ± 0.1 |
| Day 23 | 1.8 ± 0.1 | 1.6 ± 0.1 | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.8 ± 0.1 | 1.6 ± 0.1 |
| Week 14 | 1.3 ± 0.0 | 1.4 ± 0.0 | 1.3 ± 0.0 | 1.3 ± 0.0 | 1.6 ± 0.2** | 1.5 ± 0.0** |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 3 | 64 ± 2 | 61 ± 1 | 61 ± 1 | 64 ± 2 | 60 ± 2 | 56 ± 1** |
| Day 23 | 44 ± 1 | 39 ± 1* | 39 ± 1** | 38 ± 1** | 35 ± 1** | 35 ± 0** |
| Week 14 | 113 ± 6 | 113 ± 11 | 110 ± 12 | 70 ± 4** | 61 ± 3** | 50 ± 2** |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 3 | 733 ± 14 | 759 ± 16 | 779 ± 14 | 794 ± 24 | 771 ± 24 | 674 ± 15 |
| Day 23 | 496 ± 15 | 490 ± 11 | 506 ± 15 | 485 ± 15 | 456 ± 27 | 470 ± 12 |
| Week 14 | 309 ± 7 | 294 ± 4 | 293 ± 10 | 283 ± 9* | 275 ± 7** | 250 ± 6** |
| Creatine kinase (IU/L) | | | | | | |
| Day 3 | 376 ± 21 | 422 ± 50 | 476 ± 75 | 619 ± 96 | 470 ± 56 | 436 ± 62 |
| Day 23 | 301 ± 29 | 273 ± 36 | 210 ± 25* | 231 ± 26[b] | 220 ± 15 | 278 ± 29 |
| Week 14 | 126 ± 20 | 125 ± 21 | 144 ± 20 | 129 ± 20 | 179 ± 13 | 118 ± 13 |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 3 | 13 ± 1 | 12 ± 1 | 12 ± 1 | 11 ± 1 | 12 ± 0 | 11 ± 1 |
| Day 23 | 13 ± 0 | 13 ± 0 | 13 ± 0 | 12 ± 1 | 12 ± 0 | 12 ± 0 |
| Week 14 | 26 ± 1 | 24 ± 2 | 22 ± 1 | 20 ± 1** | 17 ± 1** | 17 ± 1** |
| Bile acids (µmol/L) | | | | | | |
| Day 3 | 33.8 ± 1.1 | 39.9 ± 0.9** | 47.1 ± 0.7** | 50.9 ± 1.7** | 50.4 ± 2.0** | 55.6 ± 4.1** |
| Day 23 | 32.1 ± 2.9 | 33.2 ± 1.4 | 37.7 ± 1.4* | 41.1 ± 1.0** | 43.4 ± 1.7** | 44.2 ± 1.4** |
| Week 14 | 28.6 ± 1.7 | 31.2 ± 1.3 | 32.7 ± 1.5 | 30.9 ± 0.9 | 32.4 ± 0.9* | 35.9 ± 2.2** |
| **Female** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematology | | | | | | |
| Hematocrit (%) | | | | | | |
| Day 3 | 45.5 ± 0.5 | 45.1 ± 0.4 | 45.3 ± 0.7 | 44.4 ± 0.7 | 44.0 ± 0.5 | 44.3 ± 0.5 |
| Day 23 | 48.4 ± 0.4 | 48.9 ± 0.6 | 48.1 ± 0.3 | 48.6 ± 0.7 | 48.4 ± 0.5 | 48.5 ± 0.6 |
| Week 14 | 42.1 ± 0.5 | 41.8 ± 0.4 | 42.0 ± 0.5 | 42.4 ± 0.5 | 42.5 ± 0.4 | 43.3 ± 0.4 |
| Packed cell volume (mL/dL) | | | | | | |
| Day 3 | 43.3 ± 0.5 | 43.3 ± 0.5 | 43.2 ± 0.6 | 42.3 ± 0.7 | 41.7 ± 0.5 | 42.5 ± 0.5 |
| Day 23 | 46.6 ± 0.6 | 48.3 ± 0.7 | 45.9 ± 0.4 | 47.6 ± 0.6 | 47.6 ± 0.7 | 47.9 ± 0.7 |
| Week 14 | 41.9 ± 0.3 | 41.9 ± 0.4 | 41.0 ± 0.4 | 42.5 ± 0.5 | 42.6 ± 0.4 | 43.3 ± 0.3* |

**TABLE F1**
**Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene**

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| **Female** (continued) | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematology (continued) | | | | | | |
| Hemoglobin (g/dL) | | | | | | |
| Day 3 | 13.9 ± 0.2 | 13.8 ± 0.2 | 13.9 ± 0.2 | 13.5 ± 0.3 | 13.3 ± 0.1 | 13.7 ± 0.1 |
| Day 23 | 15.5 ± 0.2 | 15.7 ± 0.2 | 15.4 ± 0.1 | 15.5 ± 0.2 | 15.6 ± 0.2 | 15.6 ± 0.2 |
| Week 14 | 14.4 ± 0.1 | 14.4 ± 0.1 | 14.3 ± 0.1 | 14.5 ± 0.1 | 14.5 ± 0.1 | 14.6 ± 0.1 |
| Erythrocytes ($10^6$/μL) | | | | | | |
| Day 3 | 6.65 ± 0.09 | 6.67 ± 0.07 | 6.70 ± 0.10 | 6.52 ± 0.13 | 6.50 ± 0.08 | 6.62 ± 0.10 |
| Day 23 | 7.42 ± 0.11 | 7.75 ± 0.13 | 7.35 ± 0.08 | 7.58 ± 0.12 | 7.65 ± 0.14 | 7.71 ± 0.15 |
| Week 14 | 6.96 ± 0.03 | 6.99 ± 0.07 | 6.83 ± 0.07 | 7.07 ± 0.08 | 7.07 ± 0.07 | 7.17 ± 0.04 |
| Reticulocytes ($10^6$/μL) | | | | | | |
| Day 3 | 0.33 ± 0.03 | 0.34 ± 0.03 | 0.33 ± 0.03 | 0.27 ± 0.02 | 0.38 ± 0.02 | 0.28 ± 0.03 |
| Day 23 | 0.14 ± 0.01 | 0.14 ± 0.01 | 0.17 ± 0.01 | 0.16 ± 0.01 | 0.15 ± 0.01 | 0.17 ± 0.02 |
| Week 14 | 0.15 ± 0.01 | 0.15 ± 0.01 | 0.12 ± 0.01 | 0.14 ± 0.01 | 0.15 ± 0.01 | 0.12 ± 0.01 |
| Nucleated erythrocytes/100 leukocytes | | | | | | |
| Day 3 | 0.40 ± 0.22 | 1.00 ± 0.21 | 0.20 ± 0.13 | 0.60 ± 0.16 | 0.70 ± 0.34 | 0.70 ± 0.26 |
| Day 23 | 0.30 ± 0.15 | 0.20 ± 0.20 | 0.20 ± 0.13 | 0.30 ± 0.21 | 0.10 ± 0.10 | 0.30 ± 0.15 |
| Week 14 | 0.70 ± 0.26 | 1.30 ± 0.40 | 0.40 ± 0.22 | 0.50 ± 0.22 | 0.30 ± 0.21 | 0.60 ± 0.22 |
| Mean cell volume (fL) | | | | | | |
| Day 3 | 65.3 ± 0.3 | 64.9 ± 0.4 | 64.4 ± 0.4 | 64.9 ± 0.3 | 64.3 ± 0.4 | 64.4 ± 0.3 |
| Day 23 | 62.8 ± 0.3 | 62.4 ± 0.4 | 62.5 ± 0.4 | 62.7 ± 0.3 | 62.4 ± 0.5 | 62.2 ± 0.3 |
| Week 14 | 60.2 ± 0.3 | 60.1 ± 0.2 | 60.0 ± 0.2 | 60.1 ± 0.3 | 60.3 ± 0.3 | 60.3 ± 0.2 |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 3 | 20.9 ± 0.1 | 20.7 ± 0.2 | 20.8 ± 0.1 | 20.7 ± 0.2 | 20.6 ± 0.1 | 20.7 ± 0.2 |
| Day 23 | 20.9 ± 0.2 | 20.2 ± 0.2 | 20.9 ± 0.3 | 20.5 ± 0.2 | 20.4 ± 0.3 | 20.2 ± 0.3 |
| Week 14 | 20.7 ± 0.1 | 20.5 ± 0.2 | 20.9 ± 0.2 | 20.5 ± 0.2 | 20.5 ± 0.2 | 20.4 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | | | | | | |
| Day 3 | 32.2 ± 0.1 | 31.8 ± 0.4 | 32.2 ± 0.1 | 32.0 ± 0.2 | 32.0 ± 0.2 | 32.3 ± 0.2 |
| Day 23 | 33.3 ± 0.3 | 32.4 ± 0.2 | 33.4 ± 0.3 | 32.6 ± 0.3 | 32.8 ± 0.3 | 32.5 ± 0.4 |
| Week 14 | 34.4 ± 0.2 | 34.2 ± 0.3 | 34.8 ± 0.3 | 34.1 ± 0.3 | 33.9 ± 0.3 | 33.8 ± 0.3 |
| Platelets ($10^3$/μL) | | | | | | |
| Day 3 | 964.0 ± 24.6 | 860.5 ± 34.5 | 922.1 ± 46.2 | 907.5 ± 26.0 | 787.4 ± 26.9** | 904.3 ± 41.7 |
| Day 23 | 1,026.6 ± 35.1 | 940.7 ± 31.3[b] | 996.6 ± 37.6 | 963.8 ± 24.0 | 990.8 ± 51.8 | 1,042.0 ± 30.1 |
| Week 14 | 620.3 ± 10.6 | 605.8 ± 7.7 | 615.8 ± 12.9 | 618.3 ± 14.2 | 640.3 ± 13.9 | 677.7 ± 8.5** |
| Leukocytes ($10^3$/μL) | | | | | | |
| Day 3 | 10.24 ± 0.46 | 9.72 ± 0.53 | 9.77 ± 0.53 | 8.57 ± 0.40* | 8.79 ± 0.41* | 8.38 ± 0.67* |
| Day 23 | 7.99 ± 0.47 | 9.53 ± 0.57 | 8.78 ± 0.54 | 8.45 ± 0.51 | 9.96 ± 0.52* | 10.60 ± 0.57** |
| Week 14 | 4.12 ± 0.46 | 2.93 ± 0.28 | 3.18 ± 0.27 | 3.57 ± 0.40 | 4.38 ± 0.70 | 4.60 ± 0.61 |
| Segmented neutrophils ($10^3$/μL) | | | | | | |
| Day 3 | 0.98 ± 0.07 | 1.13 ± 0.14 | 0.93 ± 0.09 | 0.84 ± 0.11 | 1.09 ± 0.10 | 1.09 ± 0.15 |
| Day 23 | 0.79 ± 0.07 | 0.83 ± 0.12 | 0.84 ± 0.14 | 0.78 ± 0.09 | 0.94 ± 0.19 | 0.83 ± 0.09 |
| Week 14 | 0.67 ± 0.11 | 0.48 ± 0.07 | 0.52 ± 0.04 | 0.62 ± 0.10 | 0.77 ± 0.29 | 0.73 ± 0.12 |
| 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 |

**TABLE F1**
**Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene**

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| **Female** (continued) | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematology (continued) | | | | | | |
| Lymphocytes ($10^3$/μL) | | | | | | |
| Day 3 | 8.99 ± 0.46 | 8.30 ± 0.54 | 8.55 ± 0.48 | 7.55 ± 0.38 | 7.59 ± 0.40 | 7.06 ± 0.54** |
| Day 23 | 7.03 ± 0.51 | 8.54 ± 0.51 | 7.83 ± 0.57 | 7.52 ± 0.52 | 8.82 ± 0.44* | 9.51 ± 0.52** |
| Week 14 | 3.39 ± 0.38 | 2.43 ± 0.23 | 2.62 ± 0.25 | 2.89 ± 0.30 | 3.57 ± 0.47 | 3.83 ± 0.50 |
| Monocytes ($10^3$/μL) | | | | | | |
| Day 3 | 0.21 ± 0.04 | 0.22 ± 0.07 | 0.25 ± 0.06 | 0.13 ± 0.04 | 0.05 ± 0.01** | 0.20 ± 0.04 |
| Day 23 | 0.11 ± 0.03 | 0.13 ± 0.03 | 0.05 ± 0.03 | 0.10 ± 0.04 | 0.17 ± 0.05 | 0.17 ± 0.04 |
| Week 14 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.00 |
| Basophils ($10^3$/μL) | | | | | | |
| Day 3 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.018 ± 0.012 | 0.000 ± 0.000 | 0.018 ± 0.012 | 0.008 ± 0.008 |
| Day 23 | 0.010 ± 0.010 | 0.000 ± 0.000 | 0.017 ± 0.017 | 0.000 ± 0.000 | 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 | 0.06 ± 0.03 | 0.08 ± 0.02 | 0.04 ± 0.03 | 0.06 ± 0.03 | 0.05 ± 0.02 | 0.03 ± 0.01 |
| Day 23 | 0.05 ± 0.02 | 0.03 ± 0.01 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.03 ± 0.02 | 0.09 ± 0.03 |
| Week 14 | 0.04 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.03 ± 0.02 | 0.02 ± 0.01 | 0.04 ± 0.02 |
| Clinical Chemistry | | | | | | |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 3 | 9.0 ± 0.5 | 8.4 ± 0.5 | 7.7 ± 0.3 | 7.8 ± 0.5 | 8.8 ± 0.4 | 10.5 ± 0.5 |
| Day 23 | 12.9 ± 0.6 | 12.7 ± 0.7 | 12.3 ± 0.5 | 12.1 ± 0.3 | 11.7 ± 0.5 | 11.2 ± 0.5 |
| Week 14 | 14.4 ± 0.7 | 14.5 ± 0.3 | 14.6 ± 0.5 | 15.1 ± 0.4 | 14.4 ± 0.5 | 13.5 ± 0.6 |
| Creatinine (mg/dL) | | | | | | |
| Day 3 | 0.69 ± 0.02 | 0.69 ± 0.02 | 0.64 ± 0.02 | 0.62 ± 0.01** | 0.65 ± 0.02* | 0.62 ± 0.01** |
| Day 23 | 0.72 ± 0.01 | 0.71 ± 0.01 | 0.70 ± 0.00 | 0.72 ± 0.01 | 0.73 ± 0.02 | 0.71 ± 0.01 |
| Week 14 | 0.97 ± 0.02 | 1.05 ± 0.02 | 1.01 ± 0.02 | 1.07 ± 0.02* | 1.03 ± 0.03 | 1.04 ± 0.03 |
| Total protein (g/dL) | | | | | | |
| Day 3 | 5.7 ± 0.1 | 5.7 ± 0.1 | 5.6 ± 0.0 | 5.5 ± 0.1 | 5.6 ± 0.1 | 5.7 ± 0.0 |
| Day 23 | 6.1 ± 0.1 | 6.1 ± 0.0 | 6.0 ± 0.0 | 6.1 ± 0.1 | 6.2 ± 0.1 | 6.2 ± 0.1 |
| Week 14 | 6.6 ± 0.1 | 6.7 ± 0.1 | 6.9 ± 0.1 | 6.9 ± 0.1 | 6.9 ± 0.1* | 7.1 ± 0.1** |
| Albumin (g/dL) | | | | | | |
| Day 3 | 3.6 ± 0.1 | 3.6 ± 0.1 | 3.6 ± 0.1 | 3.5 ± 0.1 | 3.6 ± 0.0 | 3.7 ± 0.1 |
| Day 23 | 3.8 ± 0.0 | 3.8 ± 0.0 | 3.7 ± 0.0 | 3.9 ± 0.1 | 3.8 ± 0.1 | 3.8 ± 0.1 |
| Week 14 | 4.2 ± 0.1 | 4.0 ± 0.1 | 4.3 ± 0.1 | 4.2 ± 0.1 | 4.3 ± 0.1 | 4.6 ± 0.1** |
| Globulin (g/dL) | | | | | | |
| Day 3 | 2.1 ± 0.0 | 2.1 ± 0.0 | 2.0 ± 0.0 | 2.1 ± 0.1 | 2.0 ± 0.1 | 2.0 ± 0.1 |
| Day 23 | 2.4 ± 0.1 | 2.4 ± 0.0 | 2.3 ± 0.0 | 2.2 ± 0.0* | 2.4 ± 0.1 | 2.4 ± 0.0 |
| Week 14 | 2.5 ± 0.1 | 2.7 ± 0.1 | 2.5 ± 0.1 | 2.7 ± 0.1 | 2.6 ± 0.1 | 2.4 ± 0.1 |
| Albumin/globulin ratio | | | | | | |
| Day 3 | 1.7 ± 0.1 | 1.8 ± 0.1 | 1.8 ± 0.1 | 1.7 ± 0.1 | 1.8 ± 0.1 | 1.9 ± 0.1 |
| Day 23 | 1.6 ± 0.0 | 1.6 ± 0.0 | 1.6 ± 0.0 | 1.8 ± 0.0 | 1.6 ± 0.1 | 1.6 ± 0.1 |
| Week 14 | 1.7 ± 0.1 | 1.5 ± 0.1 | 1.7 ± 0.1 | 1.6 ± 0.1 | 1.7 ± 0.1 | 2.0 ± 0.1 |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 3 | 50 ± 1 | 51 ± 2 | 48 ± 2 | 48 ± 2 | 47 ± 2 | 42 ± 2** |
| Day 23 | 37 ± 1 | 37 ± 1 | 34 ± 1 | 35 ± 1 | 34 ± 1 | 33 ± 1 |
| Week 14 | 80 ± 8 | 64 ± 6 | 63 ± 4 | 53 ± 4** | 53 ± 3** | 33 ± 1** |

**TABLE F1**
**Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of Cumene**

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| **Female** (continued) | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Clinical Chemistry (continued) | | | | | | |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 3 | 642 ± 18 | 636 ± 23 | 618 ± 19 | 608 ± 12 | 585 ± 15* | 474 ± 10** |
| Day 23 | 372 ± 6 | 388 ± 11 | 370 ± 8 | 362 ± 9 | 348 ± 7* | 319 ± 7** |
| Week 14 | 272 ± 9 | 275 ± 8 | 262 ± 12 | 267 ± 7 | 243 ± 7* | 207 ± 6** |
| Creatine kinase (IU/L) | | | | | | |
| Day 3 | 303 ± 26 | 291 ± 28[b] | 326 ± 26 | 314 ± 34 | 367 ± 25 | 320 ± 49 |
| Day 23 | 242 ± 28 | 233 ± 27 | 209 ± 15 | 189 ± 17[b] | 229 ± 24 | 245 ± 24 |
| Week 14 | 149 ± 22 | 123 ± 15 | 120 ± 14 | 140 ± 17 | 125 ± 20 | 104 ± 9 |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 3 | 13 ± 1 | 13 ± 0 | 13 ± 0 | 12 ± 0 | 12 ± 0 | 12 ± 0 |
| Day 23 | 12 ± 1 | 11 ± 0 | 11 ± 0 | 12 ± 0 | 11 ± 0 | 12 ± 0 |
| Week 14 | 22 ± 1 | 21 ± 1 | 19 ± 0 | 19 ± 1 | 18 ± 1** | 15 ± 1** |
| Bile acids (μmol/L) | | | | | | |
| Day 3 | 27.2 ± 1.3 | 32.6 ± 1.9* | 34.4 ± 1.8** | 38.5 ± 1.8** | 42.5 ± 0.9** | 38.0 ± 1.7** |
| Day 23 | 23.0 ± 0.7 | 27.6 ± 1.5** | 29.1 ± 0.9** | 31.5 ± 1.1** | 38.5 ± 2.9** | 38.4 ± 3.6** |
| Week 14 | 29.7 ± 2.6 | 24.3 ± 1.3 | 26.2 ± 1.6 | 32.1 ± 4.3 | 25.7 ± 2.3 | 23.8 ± 0.9 |

* Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are given as 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 Cumene[a]**

| | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematocrit (%) | 49.5 ± 0.7 | 49.7 ± 0.4 | 49.3 ± 0.5 | 50.4 ± 0.4 | 49.9 ± 0.5 | 49.4 ± 0.6 |
| Packed cell volume (mL/dL) | 49.8 ± 0.6 | 50.2 ± 0.6 | 49.2 ± 0.5 | 50.6 ± 0.4 | 50.5 ± 0.3 | 48.7 ± 0.5 |
| Hemoglobin (g/dL) | 15.6 ± 0.2 | 15.8 ± 0.2 | 15.6 ± 0.2 | 15.9 ± 0.1 | 15.8 ± 0.1 | 15.5 ± 0.2 |
| Erythrocytes ($10^6$/µL) | 9.74 ± 0.10 | 9.84 ± 0.10 | 9.64 ± 0.08 | 9.91 ± 0.08 | 9.90 ± 0.06 | 9.45 ± 0.09 |
| Reticulocytes ($10^6$/µL) | 0.17 ± 0.02 | 0.16 ± 0.02 | 0.14 ± 0.01 | 0.16 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.02 |
| Nucleated erythrocytes/ 100 leukocytes | 0.00 ± 0.00 | 0.10 ± 0.10 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.10 ± 0.10 | 0.00 ± 0.00 |
| Howell-Jolly bodies (% erythrocytes) | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.1 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 |
| Mean cell volume (fL) | 51.2 ± 0.2 | 51.0 ± 0.2 | 50.8 ± 0.2 | 51.0 ± 0.2 | 51.0 ± 0.2 | 51.7 ± 0.2 |
| Mean cell hemoglobin (pg) | 16.0 ± 0.1 | 16.1 ± 0.1 | 16.2 ± 0.1 | 16.1 ± 0.1 | 16.0 ± 0.1 | 16.4 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | 31.3 ± 0.2 | 31.6 ± 0.2 | 31.8 ± 0.2 | 31.5 ± 0.2 | 31.4 ± 0.1 | 31.9 ± 0.3 |
| Platelets ($10^3$/µL) | 724.2 ± 14.7 | 733.8 ± 9.0 | 732.1 ± 9.0 | 736.7 ± 10.5 | 760.6 ± 13.8* | 765.4 ± 7.1* |
| Leukocytes ($10^3$/µL) | 2.65 ± 0.29 | 2.92 ± 0.30 | 3.18 ± 0.43 | 3.38 ± 0.46 | 3.04 ± 0.43 | 3.02 ± 0.35 |
| Segmented neutrophils ($10^3$/µL) | 0.32 ± 0.05 | 0.34 ± 0.06 | 0.34 ± 0.05 | 0.43 ± 0.09 | 0.50 ± 0.21 | 0.41 ± 0.06 |
| 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) | 2.31 ± 0.25 | 2.52 ± 0.26 | 2.82 ± 0.39 | 2.91 ± 0.40 | 2.49 ± 0.31 | 2.51 ± 0.31 |
| Monocytes ($10^3$/µL) | 0.00 ± 0.00 | 0.03 ± 0.01* | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.04 ± 0.01* |
| Basophils ($10^3$/µL) | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.002 ± 0.002 | 0.000 ± 0.000 |
| Eosinophils ($10^3$/µL) | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.05 ± 0.02 |
| **Female** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 2 |
| Hematocrit (%) | 49.2 ± 0.8 | 50.5 ± 0.5 | 50.2 ± 0.3 | 50.1 ± 0.5 | 50.6 ± 0.6 | 49.0 ± 1.0 |
| Packed cell volume (mL/dL) | 49.6 ± 0.7 | 50.7 ± 0.5 | 50.5 ± 0.5 | 50.6 ± 0.5 | 51.0 ± 0.6 | 49.3 ± 0.5 |
| Hemoglobin (g/dL) | 15.6 ± 0.2 | 15.9 ± 0.2 | 15.9 ± 0.2 | 16.0 ± 0.2 | 15.9 ± 0.2 | 15.6 ± 0.4 |
| Erythrocytes ($10^6$/µL) | 9.56 ± 0.11 | 9.73 ± 0.09 | 9.71 ± 0.09 | 9.74 ± 0.11 | 9.75 ± 0.10 | 9.42 ± 0.11 |
| Reticulocytes ($10^6$/µL) | 0.21 ± 0.02 | 0.18 ± 0.02 | 0.22 ± 0.02 | 0.20 ± 0.02 | 0.24 ± 0.02 | 0.21 ± 0.06 |
| Nucleated erythrocytes/ 100 leukocytes | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.30 ± 0.21 | 0.10 ± 0.10 | 0.00 ± 0.00 |
| Howell-Jolly bodies (% erythrocytes) | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.1 |
| Mean cell volume (fL) | 51.9 ± 0.2 | 52.0 ± 0.0 | 52.2 ± 0.1 | 52.0 ± 0.3 | 52.3 ± 0.2 | 52.0 ± 0.0 |
| Mean cell hemoglobin (pg) | 16.4 ± 0.1 | 16.4 ± 0.1 | 16.3 ± 0.1 | 16.4 ± 0.1 | 16.3 ± 0.1 | 16.6 ± 0.2 |
| Mean cell hemoglobin concentration (g/dL) | 31.5 ± 0.1 | 31.4 ± 0.1 | 31.4 ± 0.1[b] | 31.5 ± 0.1 | 31.3 ± 0.2[b] | 31.6 ± 0.4 |
| Platelets ($10^3$/µL) | 715.2 ± 13.1 | 694.7 ± 20.6 | 718.4 ± 13.4[b] | 730.5 ± 10.6 | 754.2 ± 26.5[b] | 778.5 ± 1.5 |
| Leukocytes ($10^3$/µL) | 2.79 ± 0.41 | 3.07 ± 0.15 | 2.95 ± 0.17 | 3.18 ± 0.24 | 3.49 ± 0.24 | 2.35 ± 0.25 |
| Segmented neutrophils ($10^3$/µL) | 0.26 ± 0.03 | 0.36 ± 0.09 | 0.28 ± 0.05 | 0.30 ± 0.04 | 0.39 ± 0.07 | 0.34 ± 0.00 |
| 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) | 2.50 ± 0.39 | 2.68 ± 0.12 | 2.62 ± 0.14 | 2.83 ± 0.21 | 3.02 ± 0.20 | 1.98 ± 0.24 |
| Monocytes ($10^3$/µL) | 0.01 ± 0.01 | 0.01 ± 0.00 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.02 |
| 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) | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.06 ± 0.02 | 0.03 ± 0.01 |

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

# 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 Cumene[a]**

| | Chamber | | | | |
|---|---|---|---|---|---|
| | Control | 250 ppm | 500 ppm | 1,000 ppm | 2,000 ppm |
| **Male** | | | | | |
| n | 5 | 5 | 5 | 5 | 3 |
| Necropsy body wt | 146 ± 4 | 149 ± 4 | 148 ± 7 | 148 ± 5 | 102 ± 3** |
| Heart | | | | | |
| Absolute | 0.612 ± 0.033 | 0.566 ± 0.015 | 0.576 ± 0.029 | 0.558 ± 0.022 | 0.457 ± 0.017** |
| Relative | 4.208 ± 0.258 | 3.788 ± 0.036 | 3.893 ± 0.110 | 3.778 ± 0.046 | 4.471 ± 0.101 |
| R. Kidney | | | | | |
| Absolute | 0.568 ± 0.017 | 0.660 ± 0.022* | 0.648 ± 0.026 | 0.690 ± 0.028** | 0.563 ± 0.015 |
| Relative | 3.896 ± 0.071 | 4.413 ± 0.047** | 4.380 ± 0.033** | 4.672 ± 0.080** | 5.526 ± 0.231** |
| Liver | | | | | |
| Absolute | 6.282 ± 0.228 | 7.442 ± 0.329* | 7.544 ± 0.481* | 9.214 ± 0.411** | 8.207 ± 0.247** |
| Relative | 43.052 ± 0.696 | 49.706 ± 1.030** | 50.775 ± 1.242** | 62.329 ± 0.830** | 80.343 ± 0.194** |
| Lung | | | | | |
| Absolute | 1.068 ± 0.063 | 1.244 ± 0.110 | 1.088 ± 0.101 | 1.262 ± 0.082 | 1.070 ± 0.142 |
| Relative | 7.324 ± 0.403 | 8.344 ± 0.782 | 7.320 ± 0.456 | 8.582 ± 0.614 | 10.425 ± 1.121* |
| R. Testis | | | | | |
| Absolute | 0.918 ± 0.012 | 0.913 ± 0.069 | 0.868 ± 0.104 | 0.947 ± 0.026 | 0.630 ± 0.089* |
| Relative | 6.310 ± 0.171 | 6.081 ± 0.360 | 5.780 ± 0.537 | 6.421 ± 0.092 | 6.135 ± 0.716 |
| Thymus | | | | | |
| Absolute | 0.454 ± 0.013 | 0.459 ± 0.007 | 0.434 ± 0.023 | 0.452 ± 0.026 | 0.170 ± 0.011** |
| Relative | 3.123 ± 0.116 | 3.080 ± 0.104 | 2.932 ± 0.064 | 3.061 ± 0.130 | 1.662 ± 0.103** |
| **Female** | | | | | |
| n | 5 | 5 | 5 | 5 | 2 |
| Necropsy body wt | 120 ± 3 | 121 ± 3 | 125 ± 1 | 122 ± 3 | 93 ± 3** |
| Heart | | | | | |
| Absolute | 0.494 ± 0.010 | 0.516 ± 0.031 | 0.510 ± 0.011 | 0.498 ± 0.015 | 0.440 ± 0.030 |
| Relative | 4.132 ± 0.137 | 4.245 ± 0.205 | 4.095 ± 0.098 | 4.069 ± 0.071 | 4.718 ± 0.178 |
| R. Kidney | | | | | |
| Absolute | 0.486 ± 0.011 | 0.572 ± 0.023** | 0.594 ± 0.006** | 0.582 ± 0.015** | 0.550 ± 0.030 |
| Relative | 4.060 ± 0.102 | 4.706 ± 0.116** | 4.768 ± 0.022** | 4.761 ± 0.119** | 5.900 ± 0.142** |
| Liver | | | | | |
| Absolute | 5.004 ± 0.134 | 5.632 ± 0.163* | 6.204 ± 0.183** | 7.022 ± 0.251** | 7.030 ± 0.010** |
| Relative | 41.800 ± 1.128 | 46.390 ± 0.869* | 49.764 ± 1.089** | 57.371 ± 1.450** | 75.544 ± 2.419** |
| Lung | | | | | |
| Absolute | 0.896 ± 0.021 | 1.180 ± 0.129 | 0.978 ± 0.071 | 1.056 ± 0.140 | 0.790 ± 0.050 |
| Relative | 7.488 ± 0.216 | 9.787 ± 1.219 | 7.835 ± 0.505 | 8.592 ± 1.036 | 8.505 ± 0.797 |
| Thymus | | | | | |
| Absolute | 0.385 ± 0.017 | 0.399 ± 0.022 | 0.383 ± 0.008 | 0.357 ± 0.030 | 0.187 ± 0.007** |
| Relative | 3.223 ± 0.164 | 3.301 ± 0.230 | 3.074 ± 0.034 | 2.903 ± 0.193 | 2.007 ± 0.014** |

* Significantly different ($P \le 0.05$) from the chamber control group by Williams' or Dunnett's test
** $P \le 0.01$
[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). No data are reported for the 4,000 ppm groups due to 100% mortality.

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

| 1,000 ppm | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| **Male** | | | | | | |
| Necropsy body wt | 312 ± 8 | 313 ± 6 | 322 ± 5 | 331 ± 4 | 314 ± 5 | 323 ± 5 |
| Heart | | | | | | |
| Absolute | 0.850 ± 0.024 | 0.881 ± 0.019 | 0.888 ± 0.018 | 0.893 ± 0.015 | 0.885 ± 0.015 | 0.915 ± 0.015 |
| Relative | 2.726 ± 0.022 | 2.812 ± 0.021 | 2.754 ± 0.028 | 2.695 ± 0.030 | 2.820 ± 0.035 | 2.832 ± 0.037 |
| R. Kidney | | | | | | |
| Absolute | 0.923 ± 0.024 | 0.980 ± 0.025 | 1.010 ± 0.031*[b] | 1.059 ± 0.021** | 1.070 ± 0.017** | 1.152 ± 0.023** |
| Relative | 2.962 ± 0.032 | 3.128 ± 0.051** | 3.131 ± 0.056**[b] | 3.194 ± 0.036** | 3.411 ± 0.045** | 3.561 ± 0.029** |
| Liver | | | | | | |
| Absolute | 9.518 ± 0.327 | 10.123 ± 0.267 | 10.260 ± 0.264 | 11.170 ± 0.302** | 11.589 ± 0.282** | 12.637 ± 0.288** |
| Relative | 30.481 ± 0.348 | 32.279 ± 0.278* | 31.792 ± 0.430* | 33.660 ± 0.558** | 36.895 ± 0.563** | 39.068 ± 0.549** |
| Lung | | | | | | |
| Absolute | 1.431 ± 0.049 | 1.520 ± 0.044 | 1.523 ± 0.038 | 1.578 ± 0.027 | 1.548 ± 0.051 | 1.681 ± 0.081** |
| Relative | 4.586 ± 0.078 | 4.851 ± 0.103 | 4.724 ± 0.090 | 4.765 ± 0.078 | 4.925 ± 0.116 | 5.210 ± 0.272* |
| R. Testis | | | | | | |
| Absolute | 1.349 ± 0.034 | 1.364 ± 0.022 | 1.376 ± 0.034[b] | 1.390 ± 0.015 | 1.343 ± 0.027 | 1.399 ± 0.017 |
| Relative | 4.333 ± 0.067 | 4.360 ± 0.069 | 4.279 ± 0.059[b] | 4.198 ± 0.051 | 4.281 ± 0.077 | 4.331 ± 0.054 |
| Thymus | | | | | | |
| Absolute | 0.316 ± 0.011 | 0.326 ± 0.014 | 0.300 ± 0.020 | 0.337 ± 0.013 | 0.323 ± 0.012 | 0.316 ± 0.011 |
| Relative | 1.016 ± 0.038 | 1.042 ± 0.046 | 0.930 ± 0.056 | 1.017 ± 0.034 | 1.028 ± 0.028 | 0.978 ± 0.038 |
| **Female** | | | | | | |
| Necropsy body wt | 195 ± 2 | 190 ± 3 | 194 ± 4 | 190 ± 3 | 185 ± 3 | 187 ± 4 |
| Heart | | | | | | |
| Absolute | 0.646 ± 0.011 | 0.634 ± 0.011 | 0.633 ± 0.007 | 0.655 ± 0.016 | 0.625 ± 0.012 | 0.662 ± 0.016 |
| Relative | 3.312 ± 0.034 | 3.347 ± 0.065 | 3.248 ± 0.045 | 3.438 ± 0.045 | 3.380 ± 0.058 | 3.540 ± 0.058** |
| R. Kidney | | | | | | |
| Absolute | 0.637 ± 0.016 | 0.636 ± 0.010 | 0.649 ± 0.018 | 0.655 ± 0.017 | 0.645 ± 0.011 | 0.675 ± 0.011 |
| Relative | 3.263 ± 0.061 | 3.355 ± 0.049 | 3.322 ± 0.057 | 3.439 ± 0.057* | 3.486 ± 0.044** | 3.612 ± 0.040** |
| Liver | | | | | | |
| Absolute | 5.553 ± 0.130 | 5.669 ± 0.148 | 5.885 ± 0.204 | 5.959 ± 0.137 | 5.979 ± 0.133 | 6.923 ± 0.227** |
| Relative | 28.442 ± 0.389 | 29.858 ± 0.458 | 30.094 ± 0.634* | 31.289 ± 0.412** | 32.286 ± 0.386** | 36.958 ± 0.724** |
| Lung | | | | | | |
| Absolute | 1.093 ± 0.019 | 1.100 ± 0.030 | 1.169 ± 0.027 | 1.186 ± 0.028 | 1.098 ± 0.041 | 1.104 ± 0.023 |
| Relative | 5.606 ± 0.089 | 5.807 ± 0.168 | 6.003 ± 0.168 | 6.232 ± 0.107** | 5.920 ± 0.163 | 5.908 ± 0.100 |
| Thymus | | | | | | |
| Absolute | 0.288 ± 0.015 | 0.289 ± 0.007 | 0.280 ± 0.014 | 0.277 ± 0.011 | 0.270 ± 0.016 | 0.273 ± 0.013 |
| Relative | 1.476 ± 0.066 | 1.527 ± 0.041 | 1.432 ± 0.059 | 1.450 ± 0.043 | 1.453 ± 0.072 | 1.456 ± 0.050 |

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

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| **Male** | | | | |
| n | 5 | 5 | 5 | 5 |
| Necropsy body wt | 27.0 ± 0.5 | 27.6 ± 0.6 | 26.7 ± 0.5 | 27.0 ± 0.9 |
| Heart | | | | |
| Absolute | 0.142 ± 0.002 | 0.140 ± 0.008 | 0.132 ± 0.002 | 0.126 ± 0.007 |
| Relative | 5.268 ± 0.167 | 5.066 ± 0.282 | 4.946 ± 0.154 | 4.665 ± 0.160 |
| R. Kidney | | | | |
| Absolute | 0.234 ± 0.005 | 0.270 ± 0.007** | 0.250 ± 0.004 | 0.246 ± 0.007 |
| Relative | 8.677 ± 0.290 | 9.769 ± 0.152** | 9.355 ± 0.181 | 9.122 ± 0.099 |
| Liver | | | | |
| Absolute | 1.324 ± 0.051 | 1.520 ± 0.044** | 1.570 ± 0.022** | 1.808 ± 0.056** |
| Relative | 48.933 ± 0.989 | 54.966 ± 0.752** | 58.763 ± 1.077** | 67.040 ± 0.739** |
| Lung | | | | |
| Absolute | 0.200 ± 0.014 | 0.268 ± 0.026* | 0.198 ± 0.005 | 0.206 ± 0.019 |
| Relative | 7.394 ± 0.489 | 9.749 ± 1.067 | 7.412 ± 0.213 | 7.640 ± 0.651 |
| R. Testis | | | | |
| Absolute | 0.099 ± 0.005 | 0.103 ± 0.003 | 0.102 ± 0.002 | 0.103 ± 0.004 |
| Relative | 3.657 ± 0.223 | 3.741 ± 0.104 | 3.834 ± 0.114 | 3.823 ± 0.209 |
| Thymus | | | | |
| Absolute | 0.059 ± 0.003 | 0.051 ± 0.003 | 0.056 ± 0.001 | 0.044 ± 0.004** |
| Relative | 2.201 ± 0.147 | 1.845 ± 0.132 | 2.107 ± 0.087 | 1.643 ± 0.109* |
| **Female** | | | | |
| n | 5 | 5 | 5 | 1 |
| Necropsy body wt | 22.5 ± 0.3 | 22.6 ± 0.7 | 23.3 ± 0.7 | 23.8 |
| Heart | | | | |
| Absolute | 0.130 ± 0.003 | 0.126 ± 0.005 | 0.122 ± 0.007 | 0.130 |
| Relative | 5.768 ± 0.126 | 5.567 ± 0.174 | 5.234 ± 0.181 | 5.462 |
| R. Kidney | | | | |
| Absolute | 0.172 ± 0.004 | 0.216 ± 0.027 | 0.188 ± 0.007 | 0.210 |
| Relative | 7.641 ± 0.230 | 9.608 ± 1.357 | 8.089 ± 0.263 | 8.824 |
| Liver | | | | |
| Absolute | 1.150 ± 0.035 | 1.296 ± 0.057 | 1.458 ± 0.084** | 1.560 |
| Relative | 50.985 ± 0.981 | 57.172 ± 1.306* | 62.501 ± 2.211** | 65.546 |
| Lung | | | | |
| Absolute | 0.194 ± 0.022 | 0.216 ± 0.017 | 0.216 ± 0.015 | 0.200 |
| Relative | 8.599 ± 0.965 | 9.543 ± 0.701 | 9.271 ± 0.525 | 8.403 |
| Thymus | | | | |
| Absolute | 0.076 ± 0.006 | 0.073 ± 0.003 | 0.080 ± 0.005 | 0.062 |
| Relative | 3.379 ± 0.238 | 3.251 ± 0.192 | 3.423 ± 0.190 | 2.605 |

* 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). No data is reported for the 2,000 and 4,000 ppm groups due to 100% mortality.

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

| 1,000 ppm | Chamber Control | 62.5 ppm | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|---|
| **Male** | | | | | |
| n | 10 | 10 | 10 | 10 | 10 |
| Necropsy body wt | 38.3 ± 0.7 | 37.7 ± 0.9 | 37.0 ± 0.8 | 36.1 ± 0.8 | 35.8 ± 0.9* | 34.7 ± 0.6** |
| Heart | | | | | | |
| Absolute | 0.166 ± 0.003 | 0.167 ± 0.006 | 0.175 ± 0.005 | 0.162 ± 0.004 | 0.164 ± 0.006 | 0.152 ± 0.005 |
| Relative | 4.344 ± 0.075 | 4.437 ± 0.128 | 4.745 ± 0.136 | 4.501 ± 0.119 | 4.591 ± 0.135 | 4.387 ± 0.125 |
| R. Kidney | | | | | | |
| Absolute | 0.333 ± 0.007 | 0.332 ± 0.007 | 0.337 ± 0.007 | 0.305 ± 0.010* | 0.311 ± 0.006* | 0.285 ± 0.007** |
| Relative | 8.707 ± 0.119 | 8.828 ± 0.154 | 9.140 ± 0.204 | 8.460 ± 0.234 | 8.712 ± 0.144 | 8.229 ± 0.184 |
| Liver | | | | | | |
| Absolute | 1.531 ± 0.027 | 1.601 ± 0.049 | 1.607 ± 0.041 | 1.591 ± 0.048 | 1.705 ± 0.048* | 1.913 ± 0.070** |
| Relative | 40.048 ± 0.559 | 42.490 ± 0.894 | 43.485 ± 0.551* | 44.052 ± 0.746** | 47.668 ± 0.795** | 55.103 ± 1.501** |
| Lung | | | | | | |
| Absolute | 0.242 ± 0.006 | 0.237 ± 0.010 | 0.230 ± 0.007 | 0.219 ± 0.006 | 0.225 ± 0.010 | 0.214 ± 0.005* |
| Relative | 6.327 ± 0.138 | 6.289 ± 0.208 | 6.218 ± 0.111 | 6.090 ± 0.196 | 6.280 ± 0.178 | 6.177 ± 0.124 |
| R. Testis | | | | | | |
| Absolute | 0.117 ± 0.003 | 0.122 ± 0.001 | 0.125 ± 0.003 | 0.120 ± 0.002 | 0.117 ± 0.003 | 0.116 ± 0.002 |
| Relative | 3.058 ± 0.088 | 3.248 ± 0.079 | 3.390 ± 0.130 | 3.340 ± 0.083 | 3.293 ± 0.099 | 3.348 ± 0.054 |
| Thymus | | | | | | |
| Absolute | 0.043 ± 0.002 | 0.041 ± 0.002 | 0.043 ± 0.002 | 0.043 ± 0.003 | 0.040 ± 0.003 | 0.039 ± 0.001 |
| Relative | 1.117 ± 0.069 | 1.096 ± 0.053 | 1.160 ± 0.062 | 1.182 ± 0.076 | 1.132 ± 0.084 | 1.124 ± 0.039 |
| **Female** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 2 |
| Necropsy body wt | 32.4 ± 1.1 | 31.0 ± 1.2 | 31.4 ± 1.1 | 31.5 ± 1.1 | 29.8 ± 0.7 | 30.8 ± 1.3 |
| Heart | | | | | | |
| Absolute | 0.140 ± 0.004 | 0.139 ± 0.002 | 0.140 ± 0.003 | 0.144 ± 0.003 | 0.144 ± 0.003 | 0.135 ± 0.005 |
| Relative | 4.335 ± 0.094 | 4.527 ± 0.154 | 4.484 ± 0.117 | 4.605 ± 0.132 | 4.848 ± 0.114* | 4.404 ± 0.342 |
| R. Kidney | | | | | | |
| Absolute | 0.209 ± 0.006 | 0.202 ± 0.004 | 0.210 ± 0.003 | 0.208 ± 0.004 | 0.218 ± 0.006 | 0.230 ± 0.000 |
| Relative | 6.469 ± 0.134 | 6.563 ± 0.179 | 6.740 ± 0.181 | 6.640 ± 0.133 | 7.329 ± 0.130** | 7.492 ± 0.305** |
| Liver | | | | | | |
| Absolute | 1.453 ± 0.037 | 1.430 ± 0.047 | 1.495 ± 0.053 | 1.552 ± 0.045 | 1.593 ± 0.042* | 1.910 ± 0.110** |
| Relative | 45.016 ± 1.043 | 46.200 ± 0.778 | 47.622 ± 0.576* | 49.380 ± 0.521** | 53.510 ± 0.663** | 62.071 ± 1.054** |
| Lung | | | | | | |
| Absolute | 0.223 ± 0.006 | 0.238 ± 0.009 | 0.235 ± 0.008 | 0.229 ± 0.006 | 0.240 ± 0.008 | 0.240 ± 0.000 |
| Relative | 6.909 ± 0.159 | 7.718 ± 0.302 | 7.501 ± 0.185 | 7.316 ± 0.230 | 8.079 ± 0.265** | 7.818 ± 0.318 |
| Thymus | | | | | | |
| Absolute | 0.059 ± 0.002 | 0.050 ± 0.003* | 0.056 ± 0.002 | 0.057 ± 0.003 | 0.053 ± 0.002 | 0.052 ± 0.007 |
| Relative | 1.831 ± 0.038 | 1.592 ± 0.074 | 1.797 ± 0.055 | 1.810 ± 0.077 | 1.770 ± 0.072 | 1.669 ± 0.144 |

* 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 Cumene[a]**

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 |
| Weights (g) | | | | |
| Necropsy body wt | 312 ± 8 | 331 ± 4* | 314 ± 5 | 323 ± 5 |
| L. Cauda epididymis | 0.1805 ± 0.0072 | 0.1877 ± 0.0050 | 0.1948 ± 0.0034 | 0.1738 ± 0.0040 |
| L. Epididymis | 0.4781 ± 0.0200 | 0.4754 ± 0.0072 | 0.4843 ± 0.0079 | 0.4502 ± 0.0103 |
| L. Testis | 1.4148 ± 0.0325 | 1.4627 ± 0.0148 | 1.4299 ± 0.0288 | 1.4546 ± 0.0201 |
| Spermatid measurements | | | | |
| Spermatid heads ($10^3$/mg testis) | 127.6 ± 4.7 | 131.8 ± 4.1[b] | 128.8 ± 4.4 | 129.5 ± 5.4 |
| Spermatid heads ($10^6$/testis) | 166.8 ± 4.9 | 179.7 ± 5.6 | 170.1 ± 6.3 | 172.3 ± 8.2 |
| Spermatid count ($10^6$/cauda epididymis) | 100.28 ± 5.52 | 88.53 ± 4.55 | 95.54 ± 3.36 | 90.53 ± 2.32 |
| Epididymal spermatozoal measurements | | | | |
| Motility (%) | 85.45 ± 3.10 | 81.28 ± 2.83 | 84.10 ± 2.03 | 87.62 ± 1.30 |
| Concentration ($10^3$/mg cauda epididymal tissue) | 562.4 ± 33.2 | 475.6 ± 28.7 | 492.4 ± 22.0 | 523.2 ± 18.2 |

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

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 |
| Necropsy body wt (g) | 195 ± 2 | 190 ± 3 | 185 ± 3 | 187 ± 4 |
| Estrous cycle length (days) | 5.06 ± 0.13[b] | 4.85 ± 0.11 | 4.80 ± 0.11 | 4.90 ± 0.07 |
| Estrous stages (% of cycle) | | | | |
| Diestrus | 49.2 | 41.7 | 41.7 | 44.2 |
| Proestrus | 19.2 | 14.2 | 9.2 | 11.7 |
| Estrus | 15.8 | 25.8 | 28.3 | 25.0 |
| Metestrus | 15.8 | 18.3 | 20.8 | 19.2 |

[a] Necropsy body weight 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 weight) or Dunn's test (estrous cycle length). Evidence shows that exposed female groups 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. Exposed females spent more time in estrus and less time in proestrus than chamber control females.
[b] Estrous cycle was longer than 12 days or unclear in one animal.

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

| | Chamber Control | 250 ppm | 500 ppm | 1,000 ppm |
|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 |
| Weights (g) | | | | |
| Necropsy body wt | 38.3 ± 0.7 | 36.1 ± 0.8 | 36.3 ± 0.8 | 34.7 ± 0.6** |
| L. Cauda epididymis | 0.0196 ± 0.0010 | 0.0190 ± 0.0007 | 0.0173 ± 0.0006 | 0.0171 ± 0.0006* |
| L. Epididymis | 0.0497 ± 0.0013 | 0.0514 ± 0.0022 | 0.0493 ± 0.0010 | 0.0463 ± 0.0015 |
| L. Testis | 0.1119 ± 0.0027 | 0.1160 ± 0.0024 | 0.1116 ± 0.0026 | 0.1112 ± 0.0022 |
| Spermatid measurements | | | | |
| Spermatid heads ($10^3$/mg testis) | 184.4 ± 7.6 | 191.3 ± 7.2 | 204.5 ± 7.7 | 202.2 ± 7.3 |
| Spermatid heads ($10^6$/testis) | 18.80 ± 0.77 | 19.77 ± 0.79 | 20.67 ± 0.70 | 20.77 ± 0.88 |
| Spermatid count ($10^6$/cauda epididymis) | 18.05 ± 0.95 | 17.62 ± 1.11 | 17.53 ± 1.04 | 14.70 ± 0.87* |
| Epididymal spermatozoal measurements | | | | |
| Motility (%) | 85.44 ± 1.96 | 82.75 ± 2.41 | 79.95 ± 2.13 | 83.65 ± 2.43 |
| Concentration ($10^3$/mg cauda epididymal tissue) | 931.4 ± 52.1 | 928.6 ± 46.1 | 1,017.4 ± 56.7 | 870.9 ± 61.5 |

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' (body weights), Dunnett's (tissue weights), or Dunn's (spermatid measurements) test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Differences in epididymal spermatozoal measurements between exposed groups and the chamber control group are not significant by Dunn's test.

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

| | Chamber Control | 125 ppm | 250 ppm | 500 ppm |
|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 |
| Necropsy body wt (g) | 32.4 ± 1.1 | 31.4 ± 1.1 | 31.5 ± 1.1 | 29.8 ± 0.7 |
| Estrous cycle length (days) | 3.96 ± 0.07 | 3.93 ± 0.08 | 3.88 ± 0.05 | 4.01 ± 0.12 |
| Estrous stages (% of cycle) | | | | |
| Diestrus | 24.2 | 25.0 | 25.0 | 26.7 |
| Proestrus | 0.0 | 0.0 | 0.0 | 0.0 |
| Estrus | 51.7 | 51.7 | 52.5 | 53.3 |
| Metestrus | 24.2 | 23.3 | 22.5 | 20.0 |

[a] Necropsy body weight 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 weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the chamber control females in the relative length of time spent in the estrous stages.

# APPENDIX I
# CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

# CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

## PROCUREMENT AND CHARACTERIZATION OF CUMENE

Cumene was obtained from Sunoco, Inc. (Philadelphia, PA), in one lot (200556852) that was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were conducted by the study laboratory at Battelle Toxicology Northwest (Richland, WA), by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO), and by Chemir/Polytech Laboratories, Inc. (Maryland Heights, MO). Reports on analyses performed in support of the cumene studies are on file at the National Institute of Environmental Health Sciences.

Lot 200556852 of the chemical, a colorless liquid with a sharp, penetrating, aromatic odor, was identified as cumene by the analytical chemistry laboratory using $^{1}$H-nuclear magnetic resonance (NMR) spectroscopy and gas chromatography/mass spectrometry (GC/MS) and by Chemir/Polytech Laboratories, Inc., using infrared (IR) and $^{1}$H-NMR spectroscopy. All spectra were consistent with the literature spectra (*Aldrich*, 1993, 1997; *NIST/EPA/NIH*, 1994) of cumene. The $^{1}$H-NMR, GC/MS, and IR spectra are presented in Figures I1, I2, and I3, respectively.

The purity of lot 200556852 was determined by the analytical chemistry laboratory using gas chromatography (GC) by system A and by the study laboratory using GC by system B, C, or D (Table I1). In addition, Chemir/Polytech Laboratories, Inc., determined the moisture content of this lot by Karl Fischer titration and measured its purity by elemental analysis.

For lot 200556852, Karl Fischer titration indicated a water content ranging from approximately 50 to 220 ppm; elemental analyses for carbon and hydrogen were in agreement with the theoretical values for cumene. GC by system A detected no impurities greater than 0.05%, and the purity was determined to be approximately 100%. Using GC by system B, the area percent purity for the major cumene peak was 99.9%, and no peaks were detected with an area percent greater than 0.1%. The overall purity of lot 200556852 was determined to be greater than 99.9%.

To ensure stability, the bulk chemical was stored at controlled room temperature in the original shipping containers (55-gallon metal drums). Stability was monitored by the study laboratory during the 2-week, 3-month, and 2-year studies with GC by system B, C, or D. 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 I4. The design of the system was influenced by the relatively high boiling point of cumene (approximately 152° C) and the need to reach relatively high concentrations. Therefore, with the exception of individual chamber inlets, all vapor transport lines and dilution air were heated to the minimum temperature needed to move vapor to the chambers without condensation. A bulk supply of cumene was held in an 8-gallon stainless steel chemical reservoir and pumped through a preheater into the top of a heated glass column filled with glass beads to increase the surface area for evaporation. Heated nitrogen entering the column from below vaporized the chemical as it conveyed it out of the generator. Generator output was controlled by the delivery rate of the chemical metering pump.

Because the cumene vapor leaving the generator was above room temperature, it was transported to the exposure room at an elevated temperature to prevent condensation. In the distribution manifold cabinet, the vapor was mixed with additional heated air before it entered a short vapor distribution manifold. Concentration in the manifold was determined by the chemical pump rate, nitrogen flow rate, and dilution air flow rate, all of which

were monitored by the exposure operator. The pressure in the distribution manifold was fixed to ensure constant flow through the manifold and into the chambers as the flow of vapor to each chamber was adjusted. Electronically actuated metering valves controlled the flow to each chamber. In addition, an exposure-shutoff valve, mounted in series with each chamber-metering valve, controlled vapor delivery to each chamber. Vapor was diverted to the exposure chamber exhaust until the generation system was stable and exposures were ready to proceed. To start the exposure, the valves were opened to allow the flow of vapor to reach the chamber-metering valves and move into individual temperature-controlled delivery lines to each chamber. The vapor was then injected into the 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 condensation particle counter (Model 3022A, TSI, Inc., St. Paul, MN) was used to count the particles in all chambers before and during generation to ensure that cumene vapor, and not aerosol, was produced. No particle counts greater than 200 particles/$cm^3$ were detected.

## VAPOR CONCENTRATION MONITORING

Summaries of the chamber vapor concentrations are given in Tables I2 through I4. Concentrations of cumene in the exposure chambers were monitored by an on-line gas chromatograph using system E (Table I1). Samples were drawn from each exposure chamber approximately every 20 (2-week and 3-month studies) or 26 (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 cumene in nitrogen supplied by a permeation tube standard generator (Kin-Tek Model 491, Kin-Tek Laboratories, Inc., 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 charcoal sampling tubes (ORBO™-101, Supelco, Bellefonte, PA), extracted with toluene containing 1,2,4-trimethylbenzene as an internal standard, and analyzed by an off-line gas chromatograph using system F. The volumes of gas were sampled from each chamber at a constant flow rate ensured by a calibrated critical orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standard solutions of cumene and the internal standard (1,2,4-trimethylbenzene) in toluene.

## CHAMBER ATMOSPHERE CHARACTERIZATION

Buildup and decay rates for chamber vapor concentrations were determined with (all studies) and without (3-month and 2-year studies) 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 the 2-week studies in rats and mice with animals present, $T_{90}$ values ranged from 10 to 12 minutes, and $T_{10}$ values ranged from 10 to 11 minutes. For rats and mice in the 3-month studies, $T_{90}$ values ranged from 9 to 10 minutes without animals present and averaged 11 minutes with animals present; $T_{10}$ values ranged from 9 to 10 minutes without animals present and from 10 to 12 minutes

with animals present. For rats and mice in the 2-year studies, $T_{90}$ values ranged from 9 to 10 minutes without animals present and from 10 to 15 minutes with animals present; $T_{10}$ values ranged from 8 to 9 minutes without animals present and from 9 to 13 minutes with animals present. A $T_{90}$ value of 12 minutes was selected for all studies.

The uniformity of cumene 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 E, 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 cumene in the chambers after vapor delivery ended was determined by monitoring the postexposure vapor concentration in the 4,000 ppm chambers in the 2-week studies and the 1,000 ppm chambers in the 3-month and 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 77 minutes. In the 3-month studies, the concentration decreased to 1% of the target concentration within 25 minutes without animals present and within 28 minutes with animals present. In the 2-year studies, the concentration decreased to 1% of the target concentration within 25 (rats) and 24 (mice) minutes without animals present and within 35 (rats) and 27 (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 sorbent tubes (ORBO$^{TM}$-101, Supelco) and extracted with methylene chloride. Additional samples were collected from the generator reservoir and vapor trap, and all of the samples were analyzed using GC by system B to measure the stability and purity of cumene in the generation and delivery system. To assess whether impurities or degradation products co-eluted with cumene or the solvent, a second GC analysis of the samples was performed using a polar column capable of resolving compounds with similar boiling points and polarities (system G). The relative purity of cumene in the generator reservoir and vapor trap was measured using GC by systems C or D in conjunction with the stability and purity measurements described above by major peak comparison to the bulk test chemical.

No evidence of degradation of cumene was noted in any part of the exposure system. With the exception of one peak noted in the distribution line samples taken during the 3-month studies, no impurity peaks were resolved with an area >0.1% of the total peak area, and no additional impurities were detected with the polar GC analyses. The identity of the spurious peak was established by GC/mass spectrometry and by standard addition as 2-phenyl-2-propanol. The spectra for this peak (accounting for 0.14% and 0.10% of the total peak areas for the samples taken at the beginning and end, respectively, of the sampled generation day) closely matched a library reference spectrum for 2-phenyl-2-propanol (*NIST/EPA/NIH*, 1994) and the spectrum of a purchased standard of this chemical. The relative purity of all generator reservoir and vapor trap samples exceeded 99% compared to the bulk chemical, and these samples were 99.97% pure by area percent.

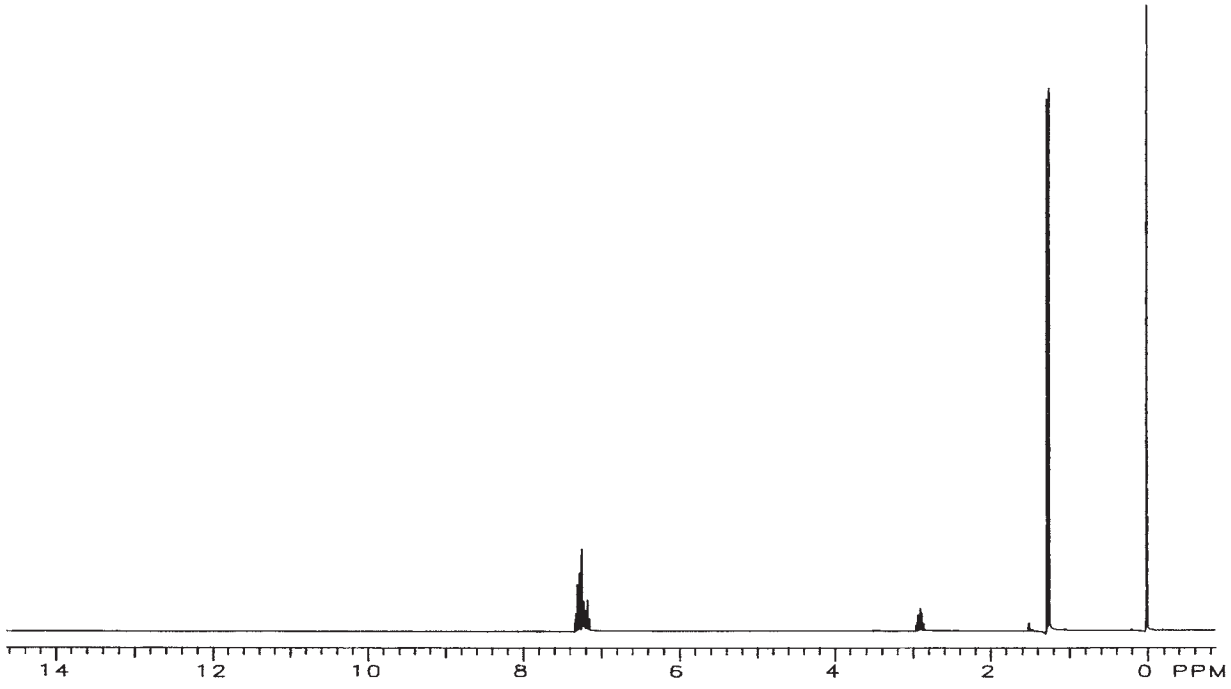


**FIGURE I1**
**$^{1}$H-Nuclear Magnetic Resonance Spectrum of Cumene**

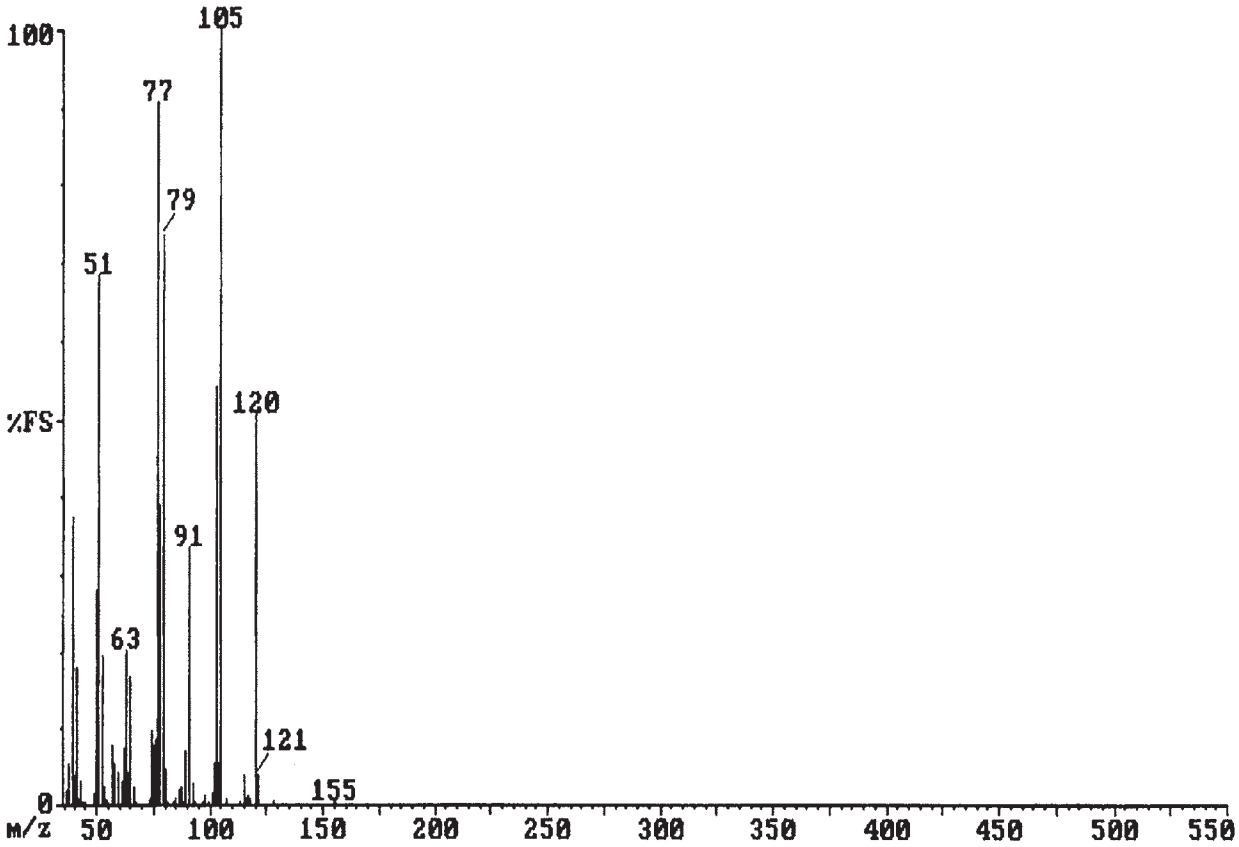


FIGURE I2
**Mass Spectrum of Cumene**

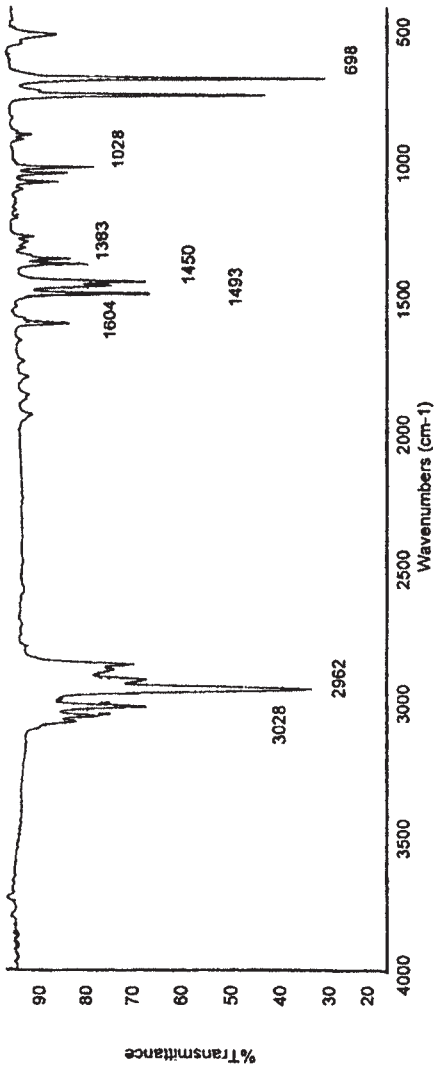


**FIGURE I3**
**Infrared Absorption Spectrum of Cumene**

**TABLE I1**
**Gas Chromatography Systems Used in the Inhalation Studies of Cumene**[a]

| Detection System | Column | Carrier Gas | Oven Temperature Program |
|---|---|---|---|
| **System A** | | | |
| Flame ionization | DBWax, 30 m × 0.53 mm, 1.0-µm film (J&W Scientific, Folsom, CA) | Helium at 10 mL/minute | 35° C for 6 minutes, then 10° C/minute to 205° C, held for 5 minutes |
| **System B** | | | |
| Flame ionization | Rtx-5, 30 m × 0.25 mm, 1.0-µm film (Restek, Bellefonte, PA) or DB-5, 30 m × 0.25 mm, 1.0-µm film (J&W Scientific) | Helium at 24 psi head pressure | 45° C for 1 minute, then 5° C/minute to 250° C |
| **System C** | | | |
| Flame ionization | Rtx-5, 30 m × 0.25 mm, 1.0-µm film (Restek) | Helium at 24 psi head pressure | 60° C for 1 minute, then 10° C/minute to 200° C |
| **System D** | | | |
| Flame ionization | Rtx-5, 30 m × 0.25 mm, 1.0-µm film (Restek) | Helium at 24 psi head pressure | 80° C for 0.5 minutes, then 8° C/minute to 135° C, then 30° C/minute to 200° C |
| **System E** | | | |
| Flame ionization | DB-5, 15 m × 0.53 mm, 1.5-µm film (J&W Scientific) | Nitrogen at 20 mL/minute | Isothermal at 85° C |
| **System F** | | | |
| Flame ionization | DB-5, 30 m × 0.53 mm, 1.5-µm film (J&W Scientific) | Helium at 6 psi head pressure | 90° C for 1 minute, then 16° C/minute to 150° C, held for 1 minute |
| **System G** | | | |
| Flame ionization | DBWax-Etr, 30 m × 0.25 mm, 0.5-µm film (J&W Scientific) | Helium at 24 psi head pressure | 45° C for 1 minute, then 5° C/minute to 250° C |

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

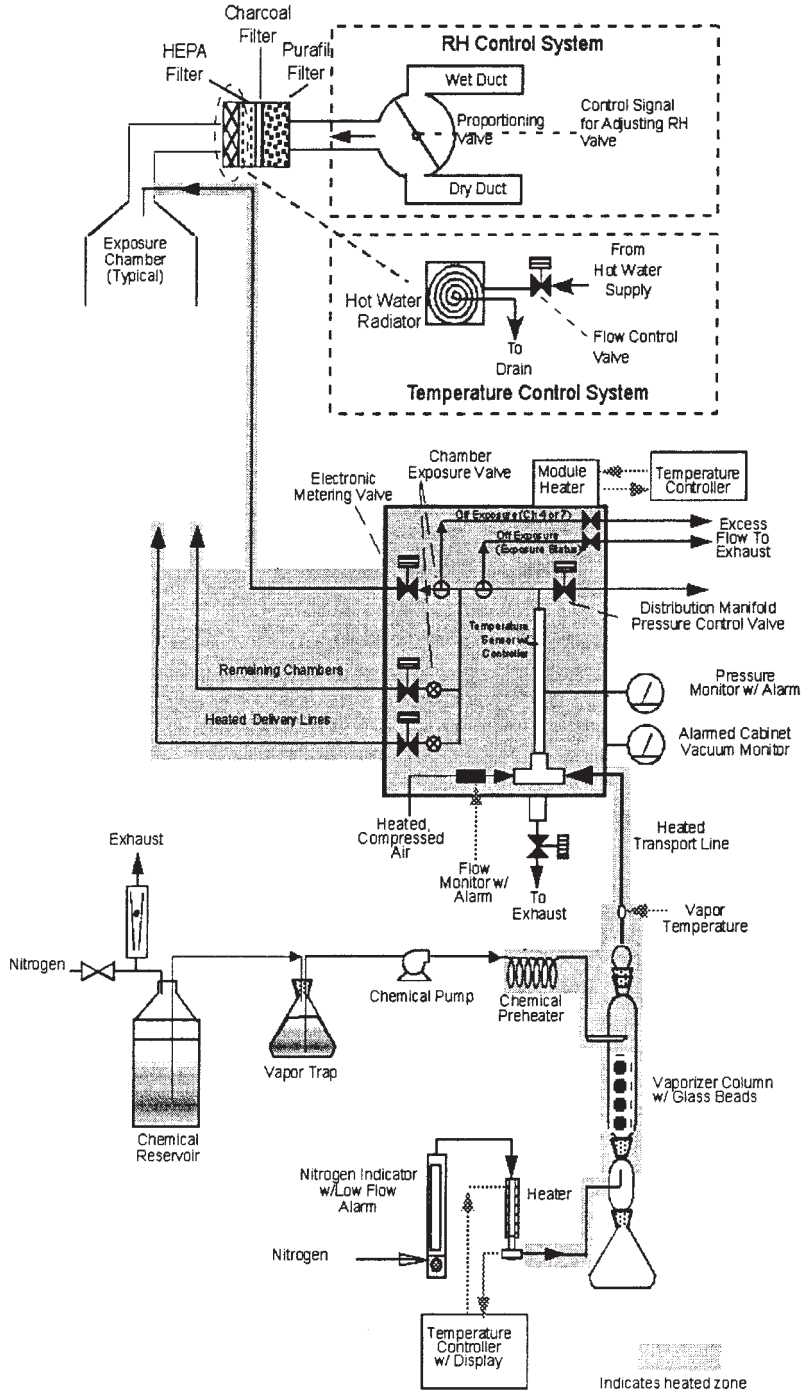


**FIGURE I4**
**Schematic of the Vapor Generation and Delivery System in the Inhalation Studies of Cumene**

**TABLE I2**
**Summary of Chamber Concentrations in the 2-Week Inhalation Studies of Cumene**

| Target Concentration (ppm) | Total Number of Readings | Average Concentration[a] (ppm) |
|---|---|---|
| **Rat Chambers** | | |
| 250 | 212 | 254 ± 4 |
| 500 | 215 | 504 ± 10 |
| 1,000 | 216 | 986 ± 30 |
| 2,000[b] | 221 | 2,047 ± 65 |
| 4,000[b] | 20 | 4,002 ± 58 |
| **Mouse Chambers** | | |
| 250 | 231 | 254 ± 4 |
| 500 | 234 | 504 ± 9 |
| 1,000[b] | 235 | 990 ± 31 |
| 2,000[b] | 20 | 2,076 ± 49 |
| 4,000[b] | 20 | 4,002 ± 58 |

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

**TABLE I3**
**Summary of Chamber Concentrations in the 3-Month Inhalation Studies of Cumene**

| Target Concentration (ppm) | Total Number of Readings | Average Concentration[a] (ppm) |
|---|---|---|
| **Rat Chambers** | | |
| 62.5 | 1,291 | 62.9 ± 1 |
| 125 | 1,298 | 125 ± 3 |
| 250 | 1,305 | 251 ± 5 |
| 500 | 1,309 | 504 ± 10 |
| 1,000 | 1,312 | 1,009 ± 16 |
| **Mouse Chambers** | | |
| 62.5 | 1,331 | 62.9 ± 1 |
| 125 | 1,338 | 125 ± 3 |
| 250 | 1,345 | 252 ± 5 |
| 500 | 1,349 | 503 ± 10 |
| 1,000 | 1,352 | 1,009 ± 16 |

[a] Mean ± standard deviation

**TABLE I4**
**Summary of Chamber Concentrations in the 2-Year Inhalation Studies of Cumene**

| Target Concentration (ppm) | Total Number of Readings | Average Concentration[a] (ppm) |
|---|---|---|
| **Rat Chambers** | | |
| 250 | 7,386 | 251 ± 5 |
| 500 | 7,440 | 502 ± 11 |
| 1,000 | 7,497 | 1,005 ± 23 |
| **Mouse Chambers** | | |
| 125 | 7,804 | 125 ± 2 |
| 250 | 7,708 | 250 ± 6 |
| 500 | 7,418 | 501 ± 13 |
| 1,000 | 7,480 | 1,007 ± 24 |

[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-Tocopherol 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) | 14.6 ± 0.63 | 13.3 – 15.7 | 23 |
| Crude Fat (% by weight) | 8.1 ± 0.27 | 7.6 – 8.6 | 23 |
| Crude Fiber (% by weight) | 9.0 ± 0.45 | 8.0 – 9.9 | 23 |
| Ash (% by weight) | 5.2 ± 0.27 | 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) | 4,794 ± 759 | 3,060 – 6,460 | 23 |
| Vitamin D (IU/kg) | 1,000[a] | | |
| α-Tocopherol (ppm) | 84.2 ± 16.60 | 52.0 – 110.0 | 15 |
| Thiamine (ppm)[b] | 7.3 ± 1.04 | 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 (%) | 1.024 ± 0.054 | 0.873 – 1.140 | 23 |
| Phosphorus (%) | 0.613 ± 0.035 | 0.556 – 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]**

| Nutrient | Mean ± Standard Deviation[b] | Range | Number of Samples |
|---|---|---|---|
| **Contaminants** | | | |
| Arsenic (ppm) | 0.32 ± 0.151 | 0.17 – 0.50 | 23 |
| Cadmium (ppm) | 0.04 ± 0.009 | 0.04 – 0.07 | 23 |
| Lead (ppm) | 0.07 ± 0.026 | 0.05 – 0.17 | 23 |
| Mercury (ppm | <0.02 | | 23 |
| Selenium (ppm) | 0.23 ± 0.052 | 0.14 – 0.36 | 23 |
| Aflatoxins (ppb) | <5.00 | | 23 |
| Nitrate nitrogen (ppm)[c] | 14.2 ± 4.13 | 6.85 – 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 ± 15 | 10 – 70c | 23 |
| Coliform (MPN/g) | 3.1 ± 0.2 | 3.0 – 3.6 | 23 |
| *Escherichia coli* (MPN/g) | <10 | | 23 |
| *Salmonella* (MPN/g) | Negative | | 23 |
| Total nitrosoamines (ppb)[e] | 4.7 ± 1.45 | 2.4 – 8.4 | 23 |
| *N*-Nitrosodimethylamine (ppb)[e] | 2.9 ± 1.37 | 1.2 – 6.9 | 23 |
| *N*-Nitrosopyrrolidine (ppb)[e] | 1.8 ± 0.78 | 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 PCB's | <0.20 | | 23 |
| Ronnel | <0.01 | | 23 |
| Ethion | <0.02 | | 23 |
| Trithion | <0.05 | | 23 |
| Diazinon | <0.10 | | 23 |
| Methyl chlorpyrifos | 0.117 ± 0.070 | 0.020 – 0.259 | 23 |
| Methyl parathion | <0.02 | | 23 |
| Ethyl parathion | <0.02 | | 23 |
| Malathion | 0.343 ± 0.486 | 0.020 – 1.850 | 23 |
| Endosulfan 1 | <0.01 | | 23 |
| Endosulfan 2 | <0.01 | | 23 |
| Endosulfane 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, five male and five female sentinel rats and mice 1 week after the start of the 3-month and 2-year studies, five male and five female sentinel rats and mice at 6, 12, and 18 months in the 2-year studies, and five males and five females from the 1,000 ppm rats and 500 ppm mice at the end of the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated; fecal samples were collected from four male and five female mice. Samples were processed appropriately and 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 |
| *M. arthritidis* | Study termination |
| *M. pulmonis* | 1 week, study termination |
| PVM | 1 week, study termination |
| RCV/SDA | 1 week, study termination |
| Sendai | 1 week, study termination |
| Immunofluorescence Assay | |
| Parvovirus | 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 week 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 |
| RCV/SDA | 18 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, study termination |
| LCM (lymphocytic choriomeningitis virus) | Study termination |
| Mouse adenoma virus-FL | Study termination |
| MHV | 1 week, study termination |
| MVM | 1 week |
| *M. arthritidis* | Study termination |
| *M. pulmonis* | 1 week, study termination |
| PVM | 1 week, study termination |
| Reovirus 3 | Study termination |
| Sendai | 1 week, study termination |
| Immunofluorescence Assay | |
| GDVII | Study termination |
| 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, 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 | 12 and 18 months, study termination |
| GDVII | 6 and 12 months |
| *Helicobacter billis* | 18 months |
| *Helicobacter hepatica* | 18 months |
| LCM | 12 months |
| MCMV | Study termination |
| MHV | Study termination |
| *M. arthritidis* | Study termination |
| Parvovirus | 6, 12, and 18 months, study termination |

## RESULTS

All results were negative.

# APPENDIX L
# CHARACTERIZATION OF K-*ras* AND *p53* MUTATIONS IN LUNG NEOPLASMS OF MICE IN THE 2-YEAR INHALATION STUDY OF CUMENE

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# CHARACTERIZATION OF K-*ras* AND *p53* MUTATIONS IN LUNG NEOPLASMS OF MICE IN THE 2-YEAR INHALATION STUDY OF CUMENE

## INTRODUCTION

Following exposure of male and female B6C3F1 mice by inhalation to 125 (females only), 250, 500, or 1,000 (males only) ppm cumene for 2 years, there were increased incidences of lung neoplasms in all groups of exposed males and females, specifically alveolar/bronchiolar adenomas and carcinomas. Cumene was not genotoxic in several studies involving bacterial and mammalian cells in culture and in *in vivo* studies involving mice (Appendix E; USEPA, 1997; HSDB, 2003). *In vitro* cell transformation assays using BALB/3T3 mouse embryo cells and unscheduled DNA synthesis assays using rat primary hepatocytes yielded conflicting results regarding a cumene effect that were not reproducible. Cumene induced a small, but significant, increase in micronucleated erythrocytes in bone marrow of male rats following intraperitoneal injection of doses ranging from 78 to 2,500 mg/kg body weight per day for 3 days (Appendix E).

Mouse alveolar/bronchiolar adenomas, which are the most common spontaneous and chemical-induced lung tumors in mice, are similar in histomorphology and molecular characteristics, including activation of the K-*ras* gene, to human adenocarcinomas (Meuwissen and Berns, 2005). The patterns of mutations in cancer genes, such as *ras* and *p53*, have been found to aid in the understanding of tumorigenesis (Harris, 1993; Maronpot *et al.*, 1995; Osada and Takahashi, 2002; Le Calvez *et al.*, 2005). For example, in some neoplasms, the profile of activating mutations in *ras* genes or inactivating mutations in the *p53* gene are specific for particular chemicals and differ from those detected in spontaneous neoplasms (Sills *et al.*, 1999, 2004).

In the present study, 52 alveolar/bronchiolar neoplasms from B6C3F1 mice exposed to cumene for 2 years were examined for mutations in exons 1 and 2 of K-*ras*, for overexpression of mutant *p53* protein using immunohistochemistry, and for mutations in exons 5 through 8 of *p53* gene. Other studies to assess changes in the K-*ras*/MAP kinase signaling pathway are in progress.

## MATERIALS AND METHODS

### *Lung Neoplasms*

Male and female B6C3F1 mice were exposed to 0, 125 (females only), 250, 500, or 1,000 (males only) ppm cumene (50 animals per group per sex) by inhalation for 6 hours per day, 5 days per week for 2 years. At necropsy, tissues were fixed in 10% neutral-buffered formalin, routinely processed, embedded in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin. Subsequently, five unstained serial sections, 10 µm thick, were prepared from paraffin blocks containing alveolar/bronchiolar adenomas or carcinomas. In order to isolate adequate amounts of DNA, neoplasms greater than 1 mm in diameter were identified for analysis. Fifty-two cumene-induced alveolar/bronchiolar neoplasms (six adenomas and 46 carcinomas), seven spontaneously occurring carcinomas, and six normal lung tissues were evaluated for K-*ras* mutations in exons 1 and 2 (codons 12, 13, and 61) and *p53* mutations in exons 5 through 8.

### *DNA Isolation, Amplification, and Cycle Sequencing*

DNA was isolated and extracted from paraffin-embedded sections containing lung neoplasms and normal lung tissue and amplified by polymerase chain reaction (PCR). Details of the use of nested primers for K-*ras* and *p53* genes have been described previously (Sills *et al.*, 1995; Lambertini *et al.*, 2005). Positive DNA controls for K-*ras* and *p53* mutations and controls lacking DNA were run with all sets of reactions. PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, Inc., Valencia, CA). The purified samples

were sequenced utilizing a cycle sequencing kit (USB Corporation, Cleveland, OH), which incorporated α-33p-dideoxynucleotide triphosphate (ddNTP) terminators (A, C, G, T) into the sequencing products. Detected mutations were confirmed by repeat analysis, starting from amplification of the original DNA extract.

### *Immunohistochemistry for p53 Protein*

Alveolar/bronchiolar adenomas and carcinomas were examined for *p53* protein expression by immunohistochemical analysis using an avidin-biotin-peroxidase detection system [VECTASTAIN Elite ABC Kit (Rabbit IgG), Vector Laboratories, Burlingame, CA]. The immunohistochemical staining for expression of mutant *p53* protein was performed as previously described (Hong *et al.*, 2000; Sills *et al.*, 2004).

## RESULTS

A higher frequency of K-*ras* mutations (45/52, 87%) was observed in the cumene-induced lung neoplasms, as compared to spontaneous lung neoplasms from control animals (historical controls, 33/117, 28%; current controls, 1/7, 14%) (Table L1). The predominant K-*ras* mutations were codon 12 G to T transversions (GTT) and codon 61 A to G transitions (CGA). These two mutations were found at frequencies of 21% (11/52) and 25% (13/52), respectively, in the neoplasms from exposed mice, compared to 0.008% (1/124) and 2% (3/124), respectively, in lung neoplasms from control mice in the historical database. Three codon 12 CGT mutations and one codon 61 CTA mutation were found in exposed groups but none in spontaneous lung neoplasms (0/124) (Table L1).

*p53* mutations were identified in 52% (27/52) of the cumene-induced lung neoplasms; none were identified in seven spontaneous carcinomas, and six were identified in normal lung tissue (Table L2). The predominant *p53* mutations were identified in exon 5 (24/27, 89%) (Table L2).

There were dose-related increases in the incidences of K-*ras* and *p53* mutations; however, a similar spectrum of both mutations was detected in cumene-induced neoplasms regardless of whether the neoplasms were adenomas or carcinomas. The *p53* protein expression was detected in 56% (29/52) of the cumene-induced lung neoplasms, mostly corresponding to *p53* mutations, and was localized to the nucleus, compared to 14% (1/7) in the spontaneously occurring neoplasms without *p53* mutation.

## DISCUSSION

A high frequency (87%) of K-*ras* mutations was identified in cumene-induced alveolar/bronchiolar neoplasms compared to that in spontaneous alveolar/bronchiolar neoplasms from control B6C3F1 mice (28% historical database; 14% current study). The predominant mutations were K-*ras* codon 12 G to T transversions (GGT to GTT, 21%) and codon 61 A to G transitions (CAA to CGA, 25%), which clearly differed from those identified in control mice (0.008% and 2%, respectively). Point mutations at codon 12 of the K-*ras* gene are activating mutations, rendering *ras* insensitive to the down-regulatory action of GTPase activating proteins, thereby locking the protein in the active state and promoting cellular transformation (Ellis and Clark, 2000). G to T transversions are commonly detected DNA base changes associated with active oxygen species and are consistent with 8-OH-G adducts produced during oxidative damage to DNA (Shigenaga and Ames, 1991; Tchou *et al.*, 1991; Janssen *et al.*, 1993). Exposure of B6C3F1 mice to ozone or vanadium pentoxide may have resulted in the generation of hydroxyl radicals, which could have induced G to T transversions at codon 12 of the K-*ras* gene (Sills *et al.*, 1995; Devereux *et al.*, 2002). Interestingly, G to T transversions in K-*ras* codon 12 are the most common mutations detected in human adenocarcinomas (Rodenhuis *et al.*, 1987). In human lung tumors, K-*ras* codon 12 G to T mutations appear to correlate with DNA adducts of benzo(a)pyrene and are associated with smoking (Reynolds *et al.*, 1987). It is possible that smoking in combination with cumene exposure in humans may have an additive effect on K-*ras* mutations.

The high frequency and pattern of K-*ras* mutations in mouse lung tumors may directly depend on the nature of the chemical carcinogen or its metabolites. In the present study, the development of lung neoplasms in the B6C3F1 mouse exposed to cumene may involve multiple carcinogenic processes, including direct DNA damage and/or indirect DNA damage such as oxidative stress. There were neoplasms without mutations of the K-*ras* gene, suggesting that other genetic events should be considered (Anderson *et al.*, 1992; Loeb, 2001).

Previous studies showed that benzene is carcinogenic and genotoxic (Gut *et al.*, 1996; Snyder and Hedli, 1996; Valentine *et al.*, 1996; Abernethy *et al.*, 2004). Side-chain oxidation of cumene is rapid and extensive and occurs in both hepatic and extrahepatic tissues, including the lung (Sato and Nakajima, 1987), with the secondary alcohol 2-phenyl-2-propanol being the principal metabolite in rats (RTI, 1989; USEPA, 1997) and humans (Lee, 1987; USEPA, 1997). The C-isopropyl bonds are readily cleaved, and the remaining electrophilic carbon moiety may form DNA adducts and cause subsequent DNA damage.

Interestingly, the *in vivo* tumor response in the present study did not show an exposure concentration-related response, suggesting that K-*ras* mutational analysis may be a more sensitive method for identifying dose response with cumene.

A high frequency (52%) of *p53* mutations were detected in cumene-induced alveolar/bronchiolar neoplasms that were correlated to *p53* protein expression (56%) by immunohistochemistry. The presence of *p53* protein expression without *p53* mutation could be due to *p53* mutations outside the regions exons 5 through 8 examined or possibly to alterations of other proteins downstream of *p53* (Greenblatt *et al.*, 1994). The predominance of cumene-induced alveolar/bronchiolar neoplasms containing *p53* mutations is consistent with these mutations, providing a selective advantage for unregulated growth and the avoidance of apoptosis (Greenblatt *et al.*, 1994; Harris, 1996; Osada and Takahashi, 2002; Rodin and Rodin, 2005). A study of aflatoxin-B1 (AFB1)-induced mouse lung tumors also found a high proportion (>70%) of tumors with *p53* accumulation and mutations (Tam *et al.*, 1999). In addition, 84% *p53* mutations were detected in lung tumors of mice exposed transplacentally to AZT (Hong *et al.*, 2007), while other studies have found a low frequency of *p53* mutations in methylene chloride-induced mouse lung tumors (Hegi *et al.*, 1993), and no *p53* mutation was present in vanadium pentoxide-induced mouse lung tumors (Devereux *et al.*, 2002). Unlike the mostly random mutation pattern for the AFB1-induced tumors (Tam *et al.*, 1999), the cumene-induced tumors had specific *p53* mutations. Only exon 5 (24/27, 89%) and exon 7 (11%) appeared to account for the *p53* mutations.

The data on K-*ras* and *p53* mutations provide evidence that these genetic alterations play an important role in cumene-induced mouse lung carcinogenesis. Additional evidence (Santillo *et al.*, 2001) showed that K-*ras* activation may play a major role in the formation of these neoplasms. Other microarray studies to investigate the role of MAP kinase signaling pathway in these neoplasms are in progress.

In conclusion, the patterns of mutations identified in the lung tumors suggest that DNA damage and genomic instability may be the contributing factors to the mutation profile and development of lung cancer in these mice. The molecular alterations identified in the cumene-induced lung neoplasms affect the same pathways as those reported in human lung cancer, suggesting that the response in the mouse may be of relevance to humans.

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**TABLE L1**
**K-*ras* Mutations in Lung Neoplasms of B6C3F1 Mice in the 2-Year Inhalation Study of Cumene[a]**

| Exposure Concentration (ppm) | Activate K-*ras* | Codon 12 (GGT) | | | | Codon 13 (GGC) | Codon 61 (CAA) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | (GAT) | (TGT) | (GTT) | (CGT) | | (CGA) | (CAT) | (CAC) | (CTA) |
| Historical controls[b] | 33/117 (28%) | 14 | 5 | 1 | 0 | 6 | 2 | 4 | 1 | 0 |
| Chamber controls | 1/7 (14%) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Cumene total | 45/52 (87%) | 6 | 5 | 11 | 3 | 4 | 13 | 0 | 2 | 1 |
| 125 | 1/4 (25%) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 250 | 10/13 (77%) | 0 | 0 | 1 | 2 | 0 | 5 | 0 | 2 | 0 |
| 500 | 17/18 (94%) | 4 | 1 | 6 | 0 | 2 | 4 | 0 | 0 | 0 |
| 1,000 | 17/17 (100%) | 2 | 3 | 4 | 1 | 2 | 4 | 0 | 0 | 1 |

[a] Only neoplasms greater than 1 mm in diameter were used; 125 ppm, females only; 1,000 ppm, males only.
[b] Historical incidences of spontaneous lung neoplasms in control B6C3F1 mice (Hong *et al.*, 2007)

**TABLE L2**
***p53* Mutations in Lung Neoplasms of B6C3F1 Mice in the 2-Year Inhalation Study of Cumene[a]**

| Exposure Concentration (ppm) | Activate *p53*[b] | IHC Positive | Exon 5 | Exon 7 |
|---|---|---|---|---|
| Chamber controls | 0/7 | 1/7 (14%) | 0 | 0 |
| Cumene total | 27/52 (52%) | 29/52 (56%) | 24 | 3 |
| 125 | 0/4 | 1/4 (25%) | 0 | 0 |
| 250 | 5/13 (38%) | 6/13 (46%) | 4 | 1 |
| 500 | 11/18 (61%) | 8/18 (44%) | 10 | 1 |
| 1,000 | 11/17 (65%) | 14/17 (82%) | 10 | 1 |

[a] Only neoplasms greater than 1 mm in diameter were used; 125 ppm, females only; 1,000 ppm, males only.
[b] No mutation detected for *p53* at exons 6 and 8 in the samples examined


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