NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 440

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF OZONE
(CAS NO. 10028-15-6)

AND

OZONE/NNK
(CAS NO. 10028-15-6/64091-91-4)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from NTP Central Data Management, NIEHS, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709 (919-541-1371).

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

OZONE

(CAS NO. 10028-15-6)

AND

OZONE/NNK

(CAS NO. 10028-15-6/64091-91-4)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

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

October 1994

NTP TR 440

NIH Publication No. 95-3371

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

C.J. Alden, Ph.D.
G.A. Boorman, D.V.M., Ph.D.
D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
S.L. Eustis, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
G.N. Rao, D.V.M., Ph.D.
B.A. Schwetz, D.V.M., Ph.D.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Battelle Pacific Northwest Laboratories
Conducted studies, evaluated pathology findings

B.J. Chou, D.V.M., Ph.D., Principal Investigator
J.A. Dill, Ph.D.
S.L. Grumbein, D.V.M., Ph.D.
P.W. Mellick, D.V.M., Ph.D.
R.A. Miller, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
S. Botts, M.S., D.V.M.
E. Gaillard, M.S., D.V.M.
W.F. MacKenzie, D.V.M., M.S.
K. Yoshitomi, D.V.M., Ph.D.

Dynamac Corporation
Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group
Evaluated slides, prepared pathology report on rats (18 August 1993)

J.C. Seely, D.V.M., Chair
PATHCO, Inc.
G.A. Boorman, D.V.M., Ph.D
National Toxicology Program
E. Gaillard, M.S., D.V.M.
Experimental Pathology Laboratories, Inc.
J.R. Hailey, D.V.M.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
J.R. Leninger, D.V.M., Ph.D.
Chemical Industry Institute of Toxicology
A. Radovsky, D.V.M., Ph.D
National Toxicology Program
K. Yoshitomi, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.

Evaluated slides, prepared pathology report on mice (26 August 1993)

P.K. Hildebrandt, D.V.M., Chair
PATHCO, Inc.
G.A. Boorman, D.V.M., Ph.D
National Toxicology Program
S. Botts, M.S., D.V.M.
Experimental Pathology Laboratories, Inc.
D. Dixon, D.V.M., Ph.D.
National Toxicology Program
F. Hahn, D.V.M., Ph.D.
Lovelace Biomedical and Environmental Research Institute
J.R. Hailey, D.V.M.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
W.F. MacKenzie, D.V.M., M.S.
Experimental Pathology Laboratories, Inc.
K.T. Morgan, Ph.D.
Chemical Industry Institute of Toxicology

Biotechnical Services, Inc.
Prepared Technical Report

D.D Lambright, Ph.D., Principal Investigator
J.R. Beverly, B.A.
G. Gordon, M.A.
T.A. King-Hunter, B.S.
T.L. Rhoades, B.S.

CONTENTS

ABSTRACT 5

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY 11

TECHNICAL REPORTS REVIEW SUBCOMMITTEE 12

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS 13

INTRODUCTION 15

MATERIALS AND METHODS 21

RESULTS 31

DISCUSSION AND CONCLUSIONS 75

REFERENCES 79

APPENDIX A Summary of Lesions in Male Rats in the 2-Year Inhalation Study of Ozone 85

APPENDIX B Summary of Lesions in Female Rats in the 2-Year Inhalation Study of Ozone 107

APPENDIX C Summary of Lesions in Male Mice in the 2-Year Inhalation Study of Ozone 125

APPENDIX D Summary of Lesions in Female Mice in the 2-Year Inhalation Study of Ozone 143

APPENDIX E Summary of Lesions in Male Rats in the 2-Year Inhalation Study of Ozone/NNK 163

APPENDIX F Summary of Lesions in Male Rats in the Lifetime Inhalation Study of Ozone 187

APPENDIX G Summary of Lesions in Female Rats in the Lifetime Inhalation Study of Ozone 205

APPENDIX H Summary of Lesions in Male Mice in the Lifetime Inhalation Study of Ozone 223

APPENDIX I Summary of Lesions in Female Mice in the Lifetime Inhalation Study of Ozone 241

APPENDIX J Genetic Toxicology 261

APPENDIX K Organ Weights and Organ-Weight-to-Body-Weight Ratios 265

APPENDIX L Chemical Characterization, Dose Formulation Studies, and Generation of Chamber Concentrations 269

APPENDIX M Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration 299

APPENDIX N Sentinel Animal Program 303

ABSTRACT

O_3

OZONE

CAS No. 10028-15-6

Chemical Formula: O_3 Molecular Weight: 48

Synonym: Triatomic oxygen

There is widespread concern over the health effects of oxidant air pollutants. The state of California and the Health Effects Institute (HEI) (a nonprofit research institute funded jointly by the U.S. Environmental Protection Agency [USEPA] and combustion engine manufacturers) nominated ozone for evaluation in long-term animal studies. The NTP study designs were a result of a series of meetings at the NIEHS with scientists from NIEHS, USEPA, and HEI, as well as experts from academic institutions working in the area of air pollutants. Male and female F344/N rats and $B6C3F_1$ mice were exposed to ozone by inhalation for 4 weeks, 2 years, or for 124 weeks (rats) or 130 weeks (mice). The oxygen used to generate the ozone was greater than 99.9% pure. Additional groups of male F344/N rats were administered injections of 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (≥ 99% pure) 3 times per week for 20 weeks and exposed to ozone by inhalation for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*.

4-Week Ozone Study in Rats

Groups of five male and five female F344/N rats were exposed to 0, 0.5, or 1.0 ppm ozone by inhalation 6 hours per day, 5 days per week, for a total of 20 days. All rats survived to the end of the study. The final mean body weights and mean body weight gains of 0.5 ppm males and females and of 1.0 ppm females were similar to those of the controls. The final mean body weight of 1.0 ppm males was 7% lower than that of the controls. Clinical findings included hypoactivity in 1.0 ppm males and females and ruffled fur in exposed groups of males.

Male and female rats exposed to 0.5 or 1.0 ppm developed multifocal lesions of the lung, which consisted of infiltration of granulocytes and macrophages with extension of the bronchial epithelium into the alveolar ducts. Female rats exposed to ozone developed minimal squamous metaplasia of the laryngeal epithelium at the base of the epiglottis.

Absolute and relative lung weights of all exposed groups of males and females were greater than those of the controls, and absolute and relative thymus weights of all exposed groups were generally lower than those of the controls.

4-Week Ozone Study in Mice

Groups of five male and five female $B6C3F_1$ mice were exposed to 0, 0.5, or 1.0 ppm ozone by inhalation 6 hours per day, 5 days per week, for a total of 20 days. All mice survived to the end of the study. The final mean body weights and body weight gains of all exposed groups of mice were less than those of the controls. Hypoactivity was observed in 1.0 ppm mice.

Male and female mice exposed to 0.5 or 1.0 ppm ozone developed patchy, multifocal lesions of the lung, which consisted of infiltration of granulocytes

and macrophages with extension of the bronchial epithelium into the alveolar ducts.

The relative lung weight of 1.0 ppm males was significantly greater than that of the controls. There were no other statistically significant differences in absolute or relative organ weights in males or females.

2-Year Ozone Study in Rats

The 2-year study was designed to include the present USEPA standard (0.12 ppm), the maximum concentration believed compatible with long-term survival (1.0 ppm), and an intermediate concentration (0.5 ppm). Groups of 50 male and 50 female F344/N rats were exposed to 0, 0.12, 0.5, or 1.0 ppm ozone by inhalation for 6 hours per day, 5 days per week, for 105 weeks.

Survival, Body Weights, and Clinical Findings

Survival of exposed groups of rats was similar to that of the controls at the end of the study. The mean body weights of 0.12 and 0.5 ppm males and females were similar to those of the controls throughout the study. The mean body weights of 1.0 ppm males and females were slightly lower than those of the controls throughout the study. Hypoactivity was observed in male and female rats exposed to ozone.

Pathology Findings

Increased incidences of ozone-induced metaplasia occurred in the nose and lung of rats exposed to 0.5 or 1.0 ppm ozone. The lesions in the nose were characterized by an increase in the number of goblet cells in the respiratory epithelium with mild squamous metaplasia of the cuboidal epithelium on the lateral wall. The increase in the number of goblet cells was found primarily in level I and II epithelium occurring along the lateral wall and on the maxilloturbinates and nasoturbinates. The metaplasia in the lung was a patchy multifocal lesion consisting of extension of the bronchial epithelium into the alveoli of the centriacinar region. This may represent more an extension of the bronchial epithelium into the pulmonary parenchyma than an actual transition of one epithelial cell type into another. There were increased incidences of squamous metaplasia at the base of the epiglottis characterized by one or more layers of flattened epithelial cells where low cuboidal cells are normally found.

There were no increases in the incidences of alveolar/bronchiolar adenoma or carcinoma in either males or females exposed to ozone.

Lifetime Ozone Study in Rats

For this study, rats were exposed to 0.5 and 1.0 ppm ozone for an additional 6 months to determine the effect of extended exposure on neoplasm incidence. Groups of 50 male and 50 female F344/N rats were exposed to 0, 0.5, or 1.0 ppm ozone by inhalation for 6 hours per day, 5 days per week, for 125 weeks.

Survival, Body Weights, and Clinical Findings

Survival rates of exposed rats were similar to those of the controls. The mean body weights of 0.5 ppm males and females were similar to those of the controls throughout the study. The mean body weights of 1.0 ppm males and females were slightly lower than those of the controls for the first two years of the study. Hypoactivity was observed in exposed groups of males and females.

Pathology Findings

Increased incidences of metaplasia occurred in the nose, larynx, and lung of rats exposed to 0.5 or 1.0 ppm ozone. The lung lesions were multifocal, centriacinar and were characterized by the presence of cuboidal epithelium (ciliated and nonciliated) along the alveolar ducts where type I epithelium is normally present. Inflammation (histiocytic infiltration) and interstitial fibrosis were observed in the lung of exposed males and females, and hyperplasia was observed in the nose of exposed male and female groups. There were no ozone-related increased incidences of neoplasms.

2-Year Ozone/NNK Study in Male Rats

An intermediate concentration of 0.5 ppm ozone was combined with exposure to two levels of a known carcinogen (0.1 and 1.0 mg NNK/kg body weight) in order to determine if ozone promotes the carcinogenic process or acts as a cocarcinogen. Groups of 48 male F344/N rats were exposed to 0 or 0.5 ppm ozone by inhalation, 6 hours per day, 5 days per week for 105 weeks. During the first 20 weeks of the study, these rats were subcutaneously injected with 0, 0.1, or 1.0 mg NNK per kg body weight in trioctanoin three times weekly.

Survival and Body Weights

Two-year survival rates of male rats were similar in all groups. Final mean body weights of all males exposed to NNK alone or NNK and ozone were similar to that of the controls, with the exception of rats exposed to 1.0 mg NNK/kg body weight and 0.5 ppm ozone. Hypoactivity was observed in males exposed to NNK and ozone, in those exposed to NNK without ozone, and in those exposed to ozone only.

Pathology Findings

Alveolar epithelial metaplasia and interstitial fibrosis occurred in all groups of rats exposed to ozone or to NNK and ozone, but not in those exposed to NNK without ozone. Increased incidences of hyperplasia occurred in groups of rats exposed to NNK or to ozone and NNK. Incidences of hyperplasia were similar among groups of rats exposed to NNK only. An increased incidence of alveolar/bronchiolar adenoma or carcinoma (combined) occurred in rats administered 1.0 mg/kg NNK, with or without ozone. The administration of ozone did not affect the occurrence of pulmonary neoplasms or nonneoplastic lesions in rats administered NNK.

2-Year Ozone Study in Mice

The 2-year study was designed to include the present USEPA standard (0.12 ppm), the maximum concentration believed compatible with long-term survival (1.0 ppm), and an intermediate concentration (0.5 ppm). Groups of 50 male and 50 female $B6C3F_1$ mice were exposed to 0, 0.12, 0.5, or 1.0 ppm ozone by inhalation for 6 hours per day, 5 days per week, for 105 weeks.

Survival, Body Weights, and Clinical Findings

Survival rates of exposed mice were generally similar to those of the controls; the 2-year survival rate of 1.0 ppm females was greater than that of the controls. The mean body weights of 0.12 and 0.5 ppm males were similar to that of the controls throughout the study; the mean body weights of 1.0 ppm males and of all exposed groups of females were generally lower than those of the controls throughout the study. Hypoactivity was observed in male and female mice exposed to ozone.

Pathology Findings

Increased incidences of metaplasia occurred in the nose and lung of mice exposed to 0.5 or 1.0 ppm ozone. The metaplasia in the nose consisted of increased thickening and extension of the squamous epithelium in the anterior portion of the nasal passage. The metaplasia in the lung consisted of extension of the bronchial epithelium into the alveoli of the centriacinar region. There were increased incidences of hyperplasia in the nose characterized by thickening of the noncuboidal (transitional) epithelium. There were increased incidences of hyperplasia in the epiglottis of female mice, a change that was characterized by a minimal increase in the thickness of the epithelium.

Incidences of alveolar/bronchiolar adenoma or carcinoma (combined) were marginally increased in 0.5 and 1.0 ppm males (0 ppm, 14/50; 0.12 ppm, 13/50; 0.5 ppm, 18/50; 1.0 ppm, 19/50) and were increased in 1.0 ppm females (6/50, 7/50, 9/49, 16/50).

Lifetime Ozone Study in Mice

For this study, mice were exposed to 0.5 and 1.0 ppm ozone for 30 months to determine the effect of extended exposure on neoplasm incidence. Groups of 50 male and 50 female $B6C3F_1$ mice were exposed to 0, 0.5, or 1.0 ppm ozone by inhalation for 6 hours per day, 5 days per week, for 130 weeks.

Survival and Body Weights

Survival rates of exposed mice were similar to those of the controls. The mean body weights of 0.5 ppm males and females were similar to those of the controls throughout the study. The mean body weights of 1.0 ppm males and females were generally lower than those of the controls throughout the study. Hypoactivity was observed in male and female mice exposed to ozone.

Pathology Findings

The incidences of alveolar/bronchiolar adenoma and carcinoma (combined) were marginally increased in exposed males (0 ppm, 16/49; 0.5 ppm, 22/49; 1.0 ppm, 21/50) and in exposed females (6/50, 8/49, 12/50).

Increased incidences of metaplasia occurred in the nose, larynx, and lung of exposed groups of males and females, and the incidences of hyperplasia were increased in the larynx and nose of exposed mice. The morphology of the lesions was similar to that seen in the 2-year study. There were no ozone-related increases in alveolar epithelial hyperplasia.

Genetic Toxicology

Ozone was mutagenic in *Salmonella typhimurium* strain TA102, with and without S9 metabolic activation.

Conclusions

Under the conditions of these 2-year and lifetime inhalation studies, there was *no evidence of carcinogenic activity** of ozone in male or female F344/N rats exposed to 0.12, 0.5, or 1.0 ppm. There was *equivocal evidence of carcinogenic activity* of ozone in male $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. There was *some evidence of carcinogenic activity* of ozone in female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma.

There was no evidence that exposure to 0.5 ppm ozone enhanced the incidence of NNK-induced pulmonary neoplasms in male rats.

Exposure of male and female rats to ozone for 2 years or 125 weeks was associated with goblet cell hyperplasia and squamous metaplasia in the nose, squamous metaplasia in the larynx, and metaplasia (extension of bronchial epithelium into the centriacinar alveolar ducts) and interstitial fibrosis in the lung. Exposure of male and female mice to ozone for 2 years or 130 weeks was associated with hyperplasia and squamous metaplasia in the nose and inflammation (histiocytic infiltration) and metaplasia (extension of bronchial epithelium into the centriacinar alveolar ducts) of the lung.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year and Lifetime Carcinogenesis and Genetic Toxicology Studies of Ozone

	Male F344/N Rats 2-Year Study	Male F344/N Rats Lifetime Study	Female F344/N Rats 2-Year Study	Female F344/N Rats Lifetime Study
Doses	0, 0.12, 0.5, or 1.0 ppm by inhalation	0, 0.5, or 1.0 ppm by inhalation	0, 0.12, 0.5, or 1.0 ppm by inhalation	0, 0.5, or 1.0 ppm by inhalation
Body weights	1.0 ppm group slightly lower than controls	1.0 ppm group lower than controls	1.0 ppm group slightly lower than controls	1.0 ppm group slightly lower than controls
Survival rates	8/49, 5/50, 7/50, 7/50	0/50, 0/50, 1/50	28/50, 24/50, 30/50, 27/50	6/50, 6/50, 7/50
Nonneoplastic effects	Nose: goblet cell hyperplasia (1/50, 4/50, 41/50, 48/50); lateral wall hyperplasia (0/50, 8/50, 50/50, 49/50) squamous metaplasia (2/50, 6/50, 36/50, 46/50) Larynx: squamous metaplasia (0/50, 2/50, 16/50, 43/50) Lung: metaplasia (0/50, 9/50, 46/50, 47/50); interstitial fibrosis (0/50, 2/50, 40/50, 44/50)	Nose: goblet cell hyperplasia (1/50, 46/49, 48/49); lateral wall hyperplasia (10/50, 48/49, 47/49); squamous metaplasia (10/50, 23/49, 40/49) Larynx: squamous metaplasia (0/50, 20/48, 43/47) Lung: metaplasia (0/50, 45/50, 50/50); histiocytic infiltration (0/50, 38/50, 49/50); interstitial fibrosis (0/50, 44/50, 50/50)	Nose: goblet cell hyperplasia (1/50, 2/50, 45/50, 50/50); lateral wall hyperplasia (2/50, 8/50, 48/50, 50/50) squamous metaplasia (2/50, 11/50, 21/50, 45/50) Larynx: squamous metaplasia (4/50, 5/50, 9/50, 43/50) Lung: metaplasia (0/50, 6/50, 48/50, 48/50); interstitial fibrosis (0/50, 0/50, 42/50, 47/50)	Nose: goblet cell hyperplasia (0/50, 47/49, 50/50); lateral wall hyperplasia (4/50, 49/49, 50/50); squamous metaplasia (5/50, 25/49, 35/50) Larynx: squamous metaplasia (2/49, 16/47, 48/50) Lung: metaplasia (0/50, 44/50, 50/50); histiocytic infiltration (0/50, 38/50, 49/50); interstitial fibrosis (0/50, 41/50, 50/50)
Neoplastic effects	None	None	None	None
Uncertain effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence		No evidence	

Summary of the 2-Year and Lifetime Carcinogenesis and Genetic Toxicology Studies of Ozone (continued)

	Male B6C3F$_1$ Mice 2-Year Study	Male B6C3F$_1$ Mice Lifetime Study	Female B6C3F$_1$ Mice 2-Year Study	Female B6C3F$_1$ Mice Lifetime Study
Doses	0, 0.12, 0.5, or 1.0 ppm by inhalation	0, 0.5, or 1.0 ppm by inhalation	0, 0.12, 0.5, or 1.0 ppm by inhalation	0, 0.5, or 1.0 ppm by inhalation
Body weights	1.0 ppm group slightly lower than controls	1.0 ppm group lower than controls	All exposed groups lower than controls	1.0 ppm group lower than controls
Survival rates	30/50, 34/50, 25/50, 27/50	14/50, 11/50, 12/50	29/50, 37/50, 33/48, 40/50	9/50, 12/50, 10/50
Nonneoplastic effects	Nose: hyperplasia (0/50, 0/50, 42/50, 50/50); squamous metaplasia (0/50, 3/50, 3/50, 36/50) Larynx: hyperplasia (1/50, 0/50, 0/50, 6/50) Lung: histiocytic infiltration (0/50, 0/50, 18/50, 31/50); metaplasia (0/50, 0/50, 48/50, 50/50)	Nose: hyperplasia (2/49, 33/48, 45/49); squamous metaplasia (1/49, 2/48, 20/49) Larynx: hyperplasia (4/49, 7/49, 15/50); squamous cell metaplasia (2/49, 1/49, 10/50) Lung: histiocytic infiltration (3/49, 40/49, 41/50); metaplasia (0/49, 48/49, 47/50)	Nose: hyperplasia (0/50, 0/50, 42/48, 50/50); squamous metaplasia (1/50, 1/50, 11/48, 36/50) Larynx: hyperplasia (0/50, 0/50, 0/49, 7/50) Lung: histiocytic infiltration (0/50, 0/50, 11/49, 42/50); metaplasia (0/50, 0/50, 43/49, 49/50)	Nose: hyperplasia (1/50, 42/49, 47/50); squamous metaplasia (2/50, 3/49, 28/50) Larynx: hyperplasia (13/50, 11/49, 24/50); squamous cell metaplasia (2/50, 2/49, 19/50) Lung: histiocytic infiltration (5/50, 39/49, 45/50); metaplasia (0/50, 43/49, 50/50)
Neoplastic effects	None	None	Lung: alveolar/ bronchiolar adenoma or carcinoma (combined) (6/50, 7/50, 9/49, 16/50)	Lung: alveolar/ bronchiolar adenoma or carcinoma (combined) (6/50, 8/49, 12/50)
Uncertain effects	Lung: alveolar/ bronchiolar adenoma or carcinoma (combined) (14/50, 13/50, 18/50, 19/50)	Lung: alveolar/ bronchiolar adenoma or carcinoma (combined) (16/49, 22/49, 21/50)	None	None
Level of evidence of carcinogenic activity	Equivocal evidence		Some evidence	
Genetic toxicology				
Salmonella typhimurium gene mutation:	Positive in strain TA102 with and without S9			

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.

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 Ozone and Ozone/NNK on November 16, 1993, 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 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.

Curtis D. Klaassen, Ph.D., Chair
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Paul T. Bailey, Ph.D.
Principal Reviewer
Environmental and Health Sciences Laboratory
Mobil Oil Corporation
Princeton, NJ

Arnold L. Brown, M.D.*
University of Wisconsin Medical School
Madison, WI

Louise Ryan, Ph.D.
Division of Biostatistics
Harvard School of Public Health and
Dana-Farber Cancer Institute
Boston, MA

Robert E. Taylor, M.D., Ph.D.
Principal Reviewer
Department of Pharmacology
Howard University College of Medicine
Washington, DC

Matthew J. van Zwieten, D.V.M., Ph.D.
Principal Reviewer
Merck Research Laboratories
West Point, PA

Jerrold M. Ward, D.V.M., Ph.D.
National Cancer Institute
Frederick, MD

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 16, 1993, the draft Technical Report on the toxicology and carcinogenesis studies of ozone and ozone/NNK received public review by the National Toxicology Program 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. G.A. Boorman, NIEHS, introduced the toxicology and carcinogenesis studies of ozone and ozone/NNK by discussing the four basic studies: (1) 4-week studies in rats and mice; (2) the standard 2-year studies in rats and mice; (3) 30-month studies in rats and mice; and (4) a 2-year cocarcinogenesis or promotion study in male rats with NNK, a known carcinogen and tobacco-specific nitrosamine. He reported on survival and body weight effects and commented on the lack of neoplastic effects in male and female rats in the 2-year and 30-month studies and on compound-related neoplastic lesions in male and female mice in the 2-year and 30-month studies. Dr. Boorman discussed factors supporting or arguing against a compound-related carcinogenic effect in male and female mice. The proposed conclusions for the studies were: *no evidence of carcinogenic activity* of ozone in male and female F/344N rats; *equivocal evidence of carcinogenic activity* of ozone in male $B6C3F_1$ mice; and *some evidence of carcinogenic activity* of ozone in female $B6C3F_1$ mice.

Dr. van Zwieten, a principal reviewer, agreed with the proposed conclusions. He suggested that the Abstract should summarize pathology findings from the 4-week studies. He added that since the report documents a comprehensive series of studies with ozone, consideration should be given to including photomicrographs of ozone-induced lesions in the respiratory tract of rodents. Dr. Boorman agreed.

Dr. Bailey, the second principal reviewer, agreed with the proposed conclusions. He said the report indicated that "hypoactivity was observed in male and female rats exposed to ozone" and asked when the hypoactivity was seen. Dr. Boorman indicated that this occurred only during exposure and immediately afterwards.

Dr. Taylor, the third principal reviewer, stated that prior to the meeting he thought *equivocal evidence of carcinogenic activity* was more appropriate for female mice based on the relatively flat dose-response curve in the lifetime ozone studies. However, after looking at the combined data from the 2-year and lifetime studies, he supported the proposed conclusions in the report for female mice as well as the other proposed conclusions. Dr. J.K. Haseman, NIEHS, said there were two primary factors supporting *some evidence of carcinogenic activity* in female mice. One was that in the 2-year study there were 16 animals with alveolar/bronchiolar adenoma or carcinoma in the female 1.0 ppm group; this incidence was more than double the maximum seen historically in inhalation study controls. Second, in the analyses of the 2-year and lifetime studies (combined), the trend and the 1 ppm effects were an order of magnitude more significant in female mice than in male mice.

Dr. Ward questioned combining the conclusions in mice particularly since the incidence of alveolar/bronchiolar adenoma or carcinoma was higher in the 2-year study than in the lifetime study. Dr. Haseman responded that the combined analyses have the advantage of using all of the data, and because survival adjusted methods are used, animals are being compared to animals of equivalent age. Dr. Y. Vostal, Environmental Health Consultants, commented that a statement in the Introduction indicating that the primary source of ozone in urban areas was automotive emissions was incorrect.

Dr. van Zwieten moved that the Technical Report on ozone and ozone/NNK be accepted with the revisions discussed and with the conclusions that there was *no evidence of carcinogenic activity* for male and female rats, *equivocal evidence of carcinogenic activity* for male mice, and *some evidence of carcinogenic activity* for female mice. Dr. Taylor seconded the motion, which was accepted by four yes votes with one abstention (Dr. Ryan).

INTRODUCTION

O_3

OZONE

CAS No. 10028-15-6

Chemical Formula: O_3 Molecular Weight: 48

Synonym: Triatomic oxygen

Chemical and Physical Properties

Ozone is a highly reactive, bluish gas with a slightly pungent odor (*Patty's Industrial Hygiene and Toxicology*, 1985). The material is highly unstable with a melting point of −192° C and a boiling point of −112° C. Ozone is approximately 1.6 times heavier than air (*Hawley's Condensed Chemical Dictionary*, 1987).

Use and Human Exposure

Ozone has been used commercially as an effective disinfectant in the treatment of wastewater, as an odor control compound for waste odors and around sewage-treatment plants, and as a disinfectant in swimming pools. Ozone is also used to bleach paper pulp and cotton fibers (Welsbach, 1980).

Ozone is the major oxidizing component in the type of air pollution known as photochemical smog. It is a highly reactive, unstable triatomic molecule that is formed naturally in the stratosphere by photodissociation of oxygen. Because the gas is very unstable and is rapidly destroyed when it reacts with components in the lower atmosphere, concentrations of ozone at ground level are usually less than 0.1 ppm. However, when ultraviolet solar radiation interacts with atmospheric pollutants (i.e., oxides of nitrogen, olefinic hydrocarbons, and aldehydes) ozone can be formed in the lower atmosphere and can contribute to the oxidant potential of polluted air. Concentrations of ozone in the lower atmosphere are variable and depend on a number of factors, including geographic location, time of year, meteorological conditions, concentrations of reactants, and the degree of activation by sunlight. In highly populated areas such as Los Angeles, CA, where particularly favorable conditions exist for the generation of atmospheric ozone, concentrations as high as 1.0 ppm have been recorded. Concentrations ranging between 0.2 and 0.5 ppm occur frequently during summer months. The U.S. Environmental Protection Agency (USEPA) standard is 0.12 ppm. The standard is attained when the expected number of days per year with maximum hourly average concentrations above 0.12 ppm is equal to or less than one (40 CFR, Part 50). Due to control efforts, the ozone concentrations in many major cities have decreased over the past 20 years, and levels above 0.5 ppm are uncommon. However, the USEPA currently estimates that more than 115 million people in the U.S. are exposed to ozone levels exceeding the USEPA standard each year (USEPA, 1986).

Absorption, Distribution, Metabolism, and Excretion

Because of the thickness and nature of the fluid lining the airways of the lung, little, if any, ozone diffuses through the air-blood interface intact. Therefore, while absorption of ozone is an important consideration, metabolism and excretion are less important concerns in ozone toxicity.

Experimental Animals

Absorption of ozone in the respiratory system depends on the morphology of the respiratory tract, oral versus oronasal breathing, depth and rate of breathing, and properties of the fluids lining the airway. Because most ozone toxicity is believed to be related to the reactive products formed by absorbed ozone, the rate of absorption and the location and thickness of the lining fluid layer is important. Dosimetric ozone studies have been summarized in an ozone criteria document (USEPA, 1986). Nasopharyngeal absorption may be particularly important in obligatory nose breathers, and this factor should be taken into account when comparing ozone toxicity in experimental animals and humans. In a study of absorption in the upper respiratory tract, Yokoyama and Frank (1972) reported a 72% ozone uptake in the nasopharynx of beagle dogs. In the lower respiratory tract, the tissue concentration is highest in the terminal bronchioles, where the mucus blanket lining the airway ends. The tissue concentration decreases rapidly distally from this location and is very low in the trachea (Miller *et al.*, 1985, 1993; Overton and Graham, 1989; Grotberg, 1990; Hu *et al.*, 1992). In both experimental animals and humans, exercise increases the dose to the centriacinar region (Miller *et al.*, 1985; Grotberg, 1990). Because ozone is highly reactive, most of it reacts with the lung lining fluid layer; there are virtually no experimental data on how deep ozone can penetrate into the lung tissue (Pryor, 1992), but it can cause peroxidation of cellular polyunsaturated fatty acids.

Humans

Most of the absorption studies in humans have used measurements of the removal of ozone from inspired air. Ozone levels were measured in a study of healthy, young, nonsmoking male volunteers, breathing through their noses only, their mouths only, or oronasally (Gerrity *et al.*, 1988). The mean extrathoracic removal efficiency was 40% and mean intrathoracic removal efficiency was 91%, suggesting that nearly all of the inspired ozone is adsorbed or reacts with lining fluids in the nasal cavity and air passages. There was a 10% greater ozone uptake by oral breathing than by nasal breathing, suggesting that oral or oronasal breathing does not pose a greater risk. Other studies have confirmed this observation (Hynes *et al.*, 1988; Adams *et al.*, 1989).

TOXICITY IN THE RESPIRATORY TRACT

Experimental Animals

The biochemical basis of ozone toxicity is not yet fully understood. Most of the toxicity is believed to be related to the ozone reaction products, including free radicals, aldehydes, hydrogen peroxide, and ozonides (Lai *et al.*, 1990; Mustafa, 1990; Pryor *et al.*, 1991). A significant portion of ozone reacts with the lipids lining the lung, and ozone will penetrate to tissues only where the fluid lining is thinner than 0.1 μm; these issues confound the determination of a relevant dose of ozone and how best to estimate total dose. There are also a variety of antioxidants that appear to protect cells from the toxic effects of ozone, including the dietary level of vitamin E (Elsayed *et al.*, 1988) and tissue glutathione levels (Boehme *et al.*, 1992); these protective mechanisms are important in evaluating the toxicity of ozone.

The LD_{50} for mice and rats exposed to ozone for 3 hours appears to be about 20 ppm (Mittler *et al.*, 1956). However, there is a four-fold increase in pulmonary lavage fluid protein in rats and mice exposed to as little as 2 ppm ozone for 4 hours (Hatch *et al.*, 1986), suggesting that exposure to 2 ppm may not be compatible with long-term survival. The cause of death in animals exposed to higher ozone concentrations appears to be related to cell death, increased permeability, and pulmonary edema. Most of the recent studies have used lower concentrations of ozone that more closely parallel levels in the environment.

The literature on the short-term toxicity of ozone is extensive and has been summarized in the most recent ozone criteria document (USEPA, 1986). All mammalian species studied react to inhaled ozone in a generally similar manner, with species variations due to physiological and structural differences of the respiratory tract. Ozone damage occurs in rodents along the entire respiratory tract, but is most severe in the terminal bronchioles (Dungworth *et al.*, 1975). Damage varies among different centriacinar regions in a single rat (Schwartz *et al.*, 1976; Boorman *et al.*, 1980). It has been shown that the severity of the damage depends on the distance of the centriacinar region from the trachea. Following acute ozone exposure in rodents and monkeys, pulmonary changes

are characterized by inflammation, increased protein in the bronchoalveolar fluid, degeneration and necrosis of airway lining cells, and increased thickness of the alveolar septa (Castleman *et al.*, 1980; Crapo *et al.*, 1984). Most of the emphasis has focused on the centriacinar region of the lung, the most sensitive site for ozone-induced toxicity. Exposure to as little as 0.1 ppm ozone is associated with flattening of the Clara cells, loss of cilia in the terminal bronchioles, and an influx of granulocytes and alveolar macrophages with a reorganization of the epithelium of the airways (Boorman *et al.*, 1980; Moore and Schwartz, 1981). Bronchiolization is also reported in nonhuman primates exposed to 0.64 ppm ozone for 1 year (Eustis *et al.*, 1981; Fujinaka *et al.*, 1985). With continued exposure, much of the inflammatory response subsides, suggesting an adaptive response to continued exposure (Schwartz *et al.*, 1976).

Ozone exposure causes alterations in the nasopharynx, larynx, and trachea. In the nasopharynx/respiratory epithelium of bonnet monkeys, there is loss of cilia and necrosis of ciliated cells (Harkema *et al.*, 1987). In rats, ozone also causes an increase in proliferation of the nonciliated epithelial cells (Johnson *et al.*, 1990).

The literature on the long-term toxicity of ozone exposure is much less extensive. In male rats exposed to 1 ppm ozone for 20 months, epithelial reorganization achieved a higher degree of structure than was observed with shorter exposure durations. The bronchial-like cells extended up to five airway generations into the gas exchange region (Pinkerton *et al.*, 1993). Thus, while the inflammatory response subsides, the morphological alterations persist. Pulmonary toxicity results in decreased host defense mechanisms, alterations in pulmonary immune mechanisms, and generally increased sensitivity to infectious agents (Gardner, 1982; Burleson *et al.*, 1989; Li and Richters, 1991; Gilmour *et al.*, 1993).

Humans

The role of ozone in human disease remains poorly defined, in part because ozone occurs in photochemical smog with a variety of other pollutants, including many particulates (Lippmann, 1989). Clinical studies have shown effects on pulmonary function in young adults, especially with exercise (Gong, 1992). Koren *et al.* (1991) have shown alterations in markers associated with pulmonary inflammation in humans exposed to ambient levels of ozone.

Systemic Toxicity

Hematologic effects have been reported in laboratory animals and humans after inhalation exposure to ozone, suggesting that ozone or ozone reaction products can cross the blood-gas barrier (USEPA, 1986). Behavioral and cardiovascular effects have also been reported and are summarized in the most recent ozone criteria document (USEPA, 1986). These effects are much more variable and less severe than the pulmonary effects that can be easily reproduced in most laboratories.

Carcinogenicity

Experimental Animals

There have been limited studies on the potential carcinogenicity of ozone in experimental animals. Hassett *et al.* (1985) reported a slight increase in the incidence of pulmonary adenomas in A/J mice following exposure to 0.31 or 0.5 ppm ozone for 6 months. There were a limited number of mice and the results are based on lung masses observed grossly. In Swiss Webster mice exposed to 0.4 or 0.8 ppm ozone for 18 weeks, there was no increase in the incidence of lung neoplasms (Last *et al.*, 1987). In a 13-month study of Wistar rats exposed to 0.05 ppm ozone, Ichinose and Sagai (1992) reported no increase in the incidence of lung neoplasms. Witschi *et al.* (1993) have reported that ozone does not affect the incidence of lung neoplasms in hamsters.

Humans

While there has been a dramatic increase in the incidence of lung neoplasms in this century, the great majority has been linked to cigarette smoking (Speizer, 1986) and there is no conclusive evidence to link ozone exposure to lung cancer in humans (Witschi, 1988).

Promotion Studies

There was no increase in the incidence of pulmonary adenomas in A/J mice treated with urethane and then exposed to 0.31 and 0.5 ppm ozone (Hassett *et al.*, 1985). There were a limited number of mice in this 6-month study. In urethane-treated A/J mice, there was no increase in the incidence of neoplasms in mice exposed to 0.4 ppm ozone but in mice exposed to 0.8 ppm there was an increase in the percentage of mice with neoplasms, but a decrease in the number of neoplasms per mouse (Last *et al.*, 1987). Ichinose

and Sagai (1992) reported an increase in the incidence of lung neoplasms in Wistar rats exposed to 0.05 ppm ozone exposure following a single injection of *N*-bis(2-hydroxypropyl) nitrosamine.

Genetic Toxicity

The genotoxicity data for ozone have been reviewed in detail by Victorin (1992). Briefly, this potent oxidizing agent is genotoxic in a variety of *in vivo* and *in vitro* bacterial, plant, and animal test systems. Many of the published test results are negative, however. The extreme reactivity, gaseous nature, and toxicity of ozone presented confounding influences in many of these tests; ozone concentrations must be carefully regulated to allow detection of mutagenicity in the absence of extreme toxicity. Also, this gas is highly labile and during prolonged exposure periods (a few hours or more), the ozone concentrations may fluctuate or drop, producing ineffective exposures. Voltage employed in the ozone generating apparatus, oxygen flow rate, and exposure time all appear to be important parameters for determining mutagenicity of ozone, particularly in bacterial studies (Dillon *et al.*, 1992). *In vitro*, ozone induced gene mutations in *Escherichia coli* K12 (Hamelin and Chung, 1974) and *Salmonella typhimurium* TA102 (Dillon *et al.*, 1992), dominant lethal mutations in *Drosophila melanogaster* (Erdman and Hernandez, 1982), chromosomal aberrations in cultured human lymphocytes (Gooch *et al.*, 1976), and fibroblasts (Guerrero *et al.*, 1979) and sister chromatid exchanges in Chinese hamster V79 cells (Shiraishi and Bandow, 1985) and human lymphocytes (Hsueh and Xiang, 1984).

In laboratory animals, exposure to ozone resulted in increased frequencies of chromosomal aberrations in lymphocytes of male and female Chinese hamsters (Tice *et al.*, 1978) and pulmonary macrophages of female F344 rats (Rithidech *et al.*, 1990), but not in lymphocytes of male C3H mice or bone marrow cells of Chinese hamsters (Gooch *et al.*, 1976). Again, in all these experiments, small differences in ozone concentration, exposure duration, and air flow may have been sufficient to produce these conflicting results. Also, lymphocytes (rather than bone marrow cells) may be a more reliable cell type to analyze for mutagenic effects of ozone due to the extreme biological reactivity of ozone.

Few *in vivo* investigations have been performed in humans exposed to ozone. Merz *et al.* (1975) reported increased frequencies of chromatid-type aberrations in the lymphocytes of six humans exposed to 0.5 ppm ozone for 6 or 10 hours, but other similar investigations yielded negative results (McKenzie *et al.*, 1977; Sarto and Viola, 1980; McKenzie, 1982). Additionally, no increase in SCEs was reported in lymphocytes of humans exposed to ozone (McKenzie *et al.*, 1977; Guerrero *et al.*, 1979; McKenzie, 1982). Interpretation of these human studies is made difficult by incomplete data presentations, lack of statistical analyses, inadequate number of study participants, inappropriate control subjects, or lack of attention to confounding factors such as additional exposures to hazards in the workplace or smoking history. Therefore, the genetic effects of ozone exposure in humans have not yet been determined.

Study Rationale

Growing concern over the health effects of oxidant air pollutants has stimulated considerable research. Because available literature was considered insufficient, the state of California and the Health Effects Institute (HEI) (a nonprofit research institute funded jointly by the USEPA and combustion engine manufacturers) nominated ozone for evaluation in long-term animal studies.

The standard 2-year studies were designed to include the present USEPA standard (0.12 ppm), the maximum concentration believed compatible with long-term survival (1 ppm), and an intermediate concentration (0.5 ppm). At the time the study designs were being considered, diesel exhaust studies in rodents had demonstrated that a majority of the neoplasms occurred after 24 months of exposure. Therefore, a second study with a 30-month exposure of the two highest concentrations was included. It was also recognized that ozone, while not acting as a direct carcinogen, could have important consequences if it promotes the carcinogenic process or acts as a cocarcinogen. Therefore, a third study was included in which male rats were exposed to an intermediate ozone concentration (0.5 ppm) and two levels (0.1 and 1.0 mg/kg) of a known pulmonary carcinogen 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) administered three times per week for 20 weeks. This tobacco-specific nitrosamine was considered a relevant carcinogen for people exposed to ozone since much is known about the carcinogenesis of NNK.

It was recognized that carcinogenicity was only one of the important endpoints of concern to policy makers, but to date most of the toxicity studies used relatively short exposure periods. Therefore, additional rats were added to the exposure chambers of the NTP studies for individual investigator-initiated research. These studies on pulmonary function, structure, and biochemistry were managed and supported by the Health Effects Institute (HEI).

Twenty months was selected as the maximum exposure because naturally occurring degenerative and neoplastic processes would not cause significant confounding problems for the investigators. Since only 164 rats were available for the nine studies supported by HEI, there was significant sharing of animals and tissues between investigators which, while limiting, did provide comparable data from the same study animals.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Ozone

Ultra-high purity compressed oxygen for the generation of ozone was obtained in nine lots. Lots 12636-11 and 12821-24 were manufactured by A.L. Welding Compressed Gases (Kennewick, WA). Lot 12636-11 was used throughout the 4-week studies and for part of the 2-year studies, and lot 12821-24 was used for part of the lifetime studies. Lot 12636-58 was manufactured by Alphagaz Specialty Gases, Division of Liquid Air Corporation (Denver, CO), and it was used for part of the 2-year and lifetime studies. Lots 12733-38, 12733-81, 12733-115, 12733-121, and 12733-142 were manufactured by Scott Specialty Gases (Fremont, CA), and were used for part of the 2-year and lifetime studies. Lot 12821-7 was manufactured by Linde Gases (Torrance, CA), and it was used for part of the 2-year and lifetime studies.

A certification of oxygen purity was obtained from each of the vendors, which showed that the supplied compressed oxygen purity was greater than or equal to 99.9%. Oxygen purity was acceptable for the studies.

4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone

The 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) was obtained from Chemsyn Science Laboratories (Lenexa, KS) in one lot (86-034-01-06). Identity, purity, and stability analyses were conducted by Research Triangle Institute (RTI) (Research Triangle Park, NC). Reports on analyses performed in support of the NNK studies are on file at the National Institute of Environmental Health Sciences (NIEHS). The methods and results of these studies are detailed in Appendix L.

The chemical, a yellow crystalline solid, was identified as NNK by infrared, ultraviolet/visible, nuclear magnetic resonance, and mass spectroscopy. The purity was determined by Karl Fischer water analysis, thin-layer chromatography, and high-performance liquid chromatography. Karl Fischer water analysis indicated 0.57% ± 0.01% water. Thin-layer chromatography by two systems indicated one spot and no impurities. High-performance liquid chromatography using two systems revealed no impurities, and separated the two geometric isomers E (88%) and Z (12%). The overall purity was determined to be greater than 99%. Subsequent purity analyses performed by the study laboratory using gas chromatography methods also found the overall purity to be greater than 99%. Stability studies of the bulk chemical were performed by RTI, using high-performance liquid chromatography. NNK was determined to be stable as a bulk chemical for at least 2 weeks when stored in the dark at temperatures of up to 26° C. To ensure stability, the bulk chemical was stored in the original container under a nitrogen blanket protected from light at approximately 5° C.

Trioctanoin

Trioctanoin was obtained from Eastman Kodak Company (Rochester, NY) in one lot, which was assigned lot number MO61289. Midwest Research Institute (MRI), (Kansas City, MO) had identified the chemical, a light yellow transparent liquid, as trioctanoin by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure of trioctanoin.

The purity was determined by Karl Fisher water analysis; elemental analysis; titrations for acid value, saponification value, and ester value; thin-layer chromatography; and gas chromatography. Karl Fischer water analysis indicated less than 0.1% water. Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for trioctanoin. From the titration results, a purity of 93% of the theoretical value was determined. Thin-layer chromatography indicated a major band and a minor and three trace impurities. Analysis by gas chromatography indicated a major peak and several impurity peaks with a cumulative area of approximately 7%

relative to the major peak. The largest impurity (5.1%) was identified by gas chromatography as dioctanoin. No attempt was made to determine the relative amounts of the two isomers. The study laboratory analyzed the bulk chemical for peroxide content. All of the trioctanoin used for dose preparation was found to have a peroxide content of less than 3 mEq/kg. Stability studies of the bulk chemical were performed by MRI, using gas chromatography. Trioctanoin was determined to be stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored in containers with a nitrogen headspace at room temperature protected from light.

Preparation and Analysis of Dose Formulations

NNK/Trioctanoin

Dose formulations (NNK in trioctanoin) were prepared every 3 weeks by mixing NNK with trioctanoin (Table L1). Stability analysis of the 0.1 mg/g dose formulation was performed by high-performance liquid chromatography. Stability was confirmed for 3 weeks when stored at room temperature. Periodic analyses of the dose formulations were conducted at the study laboratory using high-performance liquid chromatography. Dose formulations were analyzed at the start, middle and end of the 20-week NNK exposure period. All dose formulations used for the study were within specifications except for the initial 0.1 mg/mL formulation (approximately 78% of the target), which was discarded and replaced by a sample within specifications. All animal room samples were within 10% of the target concentrations (Table L2).

Generation and Monitoring of Chamber Concentrations

Ozone gas was generated from greater than 99.9% pure oxygen using a silent arc (corona) discharge ozonator (Model O3V5-O, OREC, Phoenix, AZ). The concentration in each chamber was controlled by manually adjusting the individual chamber metering valves. Detailed descriptions of the inhalation chambers are contained in Appendix L.

Chamber concentrations were monitored using an ultraviolet spectrophotometric analyzer (Dasibi Model 1003-AH or Dasibi Model 1003-PC systems) (Glendale, CA). For both monitoring systems, air sampled at each location was transported to the monitor by transfer lines of Teflon® tubing. Samples were directed to the ozone monitor through a set of eight computer-controlled, multiplexed Teflon valves. A sampling rate of 4 minutes per port assured that all ports were sampled approximately twice per hour. Each on-line monitor was calibrated by correlating the analog output of the on-line monitor with concentrations obtained using an independently calibrated, portable ozone monitor (Dasibi Model 1003-AH).

The buildup of vapor concentration in the chamber at the beginning of exposure to 90% of its final stable concentration (T_{90}) and the decay of concentration at the end of exposure to 10% (T_{10}) were measured prior to the start of each study in chambers with a full complement of mature F344/N rats and $B6C3F_1$ mice. These tests were done in conjunction with the prestart tests for the 4-week, 2-year, and lifetime ozone studies. The measurements were repeated once after the start of the 4-week, 2-year, and lifetime studies. At a chamber airflow rate of 15 air changes/hour, the theoretical values for T_{90} and T_{10} are both approximately 12.5 minutes. A T_{90} value of 30 minutes was used based on the experimental data. The T_{10} value ranged from 5 to 11 minutes.

Tests with ozone in a standard H-2000 chamber with animals present and a standard fresh air flow rate of 15 air changes per hour indicated that acceptable uniformity of the test article was not achievable. Concentration uniformity was improved by mixing the air within the chamber with enough energy through recycling that the rate of depletion of ozone was limited primarily by the ability of the animals or other surfaces to react with the chemical and not by diffusion of the chemical within the chamber. This was accomplished by using a recirculation device that increased the velocity of the air movement (Figure L6). Uniformity of ozone concentration in the exposure chambers was measured once during the 4-week studies and quarterly during the 2-year and lifetime studies. The usual criteria for between-port variance is less than or equal to 5%. While the majority of the determinations were within this range, some exceeded this value, and 10.1% was the maximum value found.

Summaries of the chamber concentrations in the 4-week and 2-year ozone studies, the ozone/NNK study, and the lifetime ozone studies are presented in Tables L3 through L6. The monthly mean exposure concentrations are presented in Figures L7 through L18.

4-Week Ozone Studies

The NTP study designs were a result of a series of meetings at the National Institute of Environmental Health Sciences (NIEHS) with scientists from NIEHS, the United States Environmental Protection Agency (USEPA), and the Health Effects Institute (HEI), as well as experts from academic institutions working in the area of air pollutants.

The 4-week ozone studies were conducted to characterize ozone toxicity, identify target organs, establish the differences between the sexes in sensitivity to ozone exposure, and to determine the appropriate concentrations to be used in the 2-year and lifetime studies.

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Simonsen Laboratories (Gilroy, CA). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 13 days before exposure began. Before the beginning of the studies, two male and two female rats and mice were randomly selected for health evaluations. Three weeks after receipt, serologic analyses were performed on five male and five female rats and mice; these animals were not a part of the 4-week ozone study and were maintained in control chambers. Sentinel animal analyses were performed according to the protocols of the NTP Sentinel Animal Program (Appendix N).

Groups of five male and five female rats and mice were exposed to ozone at concentrations of 0, 0.5, or 1.0 ppm. Animals were in the chambers for 12 minutes before T_{90} was reached; thus, animals were exposed 6 hours and 12 minutes per day (excluding weekends) for 20 exposure days during a 4-week period. Feed and water were available *ad libitum*, except during exposure periods. Rats and mice were housed individually following the quarantine period. Clinical findings were recorded daily for rats and mice. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

A necropsy was performed on all animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic evaluation were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, imbedded in paraffin, sectioned to a thickness of 6 μm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all control and 1.0 ppm rats and mice. If a lesion was observed in the nose, larynx, lung, mediastinal or bronchial lymph nodes, or thymus, that organ was examined at the 0.5 ppm level also. Table 1 lists the tissues and organs examined.

2-Year and Lifetime Ozone Studies

Study Design

For the 2-year studies, groups of 50 male and 50 female rats and mice were exposed to ozone at concentrations of 0, 0.12, 0.5, or 1.0 ppm. Rats and mice were exposed for 6 hours per day, 5 days per week; at the beginning of each exposure period, rats and mice were in the chambers for approximately 30 minutes more to allow chamber exposure concentrations to reach T_{90}. Rats and mice were exposed in this manner for 105 weeks. In lifetime studies, groups of 50 male and 50 female rats and mice were exposed to ozone concentrations of 0, 0.5, or 1.0 ppm for 125 weeks (rats) or 130 weeks (mice).

Source and Specification of Animals

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Simonsen Laboratories (Gilroy, CA) for use in the 2-year and lifetime ozone studies. Rats were quarantined for 14 days and mice for 21 days before the beginning of the studies. Five male and five female rats and three male and two female mice were selected for bacterial culture and selected histopathology prior to the beginning of the studies. Approximately 3 weeks after receipt, serology samples were collected for viral screening from up to seven male and seven female rats and two male and three female mice. Rats and mice were approximately 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 N).

Animal Maintenance

All animals were housed individually. Water was available *ad libitum*, and feed was available *ad libitum* except during exposure periods. Cage units were rotated vertically (2-year studies) or horizontally (lifetime studies) within each chamber weekly. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is found in Appendix M.

Clinical Examinations and Pathology

All animals were observed twice daily for moribundity and mortality. Body weights were recorded initially, weekly for the first 13 weeks, monthly through week 92 (2-year studies) or week 91 (lifetime studies), then every 2 weeks until the end of the study. Clinical observations were made at 4-week intervals until the final 13 weeks of exposure, when they were recorded every 2 weeks.

A complete necropsy and microscopic examination were performed on all animals. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. Tissues examined microscopically are listed in Table 1.

2-Year Ozone/NNK Study

Study Design

Groups of 48 male rats were exposed to ozone at concentrations of 0 or 0.5 ppm, 6 hours per day, 5 days per week (exclusive of holidays) for 105 weeks. The same groups of 48 rats were injected subcutaneously with trioctanoin alone or with 0.1 or 1.0 mg 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in trioctanoin per kg body weight three times weekly for the first 20 weeks of the study. At the beginning of each exposure period, rats were in the chambers for approximately 30 minutes to allow chamber exposure concentrations to reach T_{90}.

Source and Specification of Animals

Male F344/N rats were obtained from Simonsen Laboratories (Gilroy, CA) for use in the 2-year ozone/NNK study. Rats were quarantined for 12 days before the beginning of the studies. Ten male rats were selected for bacterial culture and selected histopathology prior to the beginning of the study. Twenty-one days after receipt, serology samples were collected from 10 rats for viral screening. Rats were approximately 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 N).

Animal Maintenance

Rats were housed individually. Water was available *ad libitum*, and feed was available *ad libitum* except during exposure periods. Cage units were rotated vertically within each chamber weekly. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix M.

Clinical Examinations and Pathology

All animals were observed twice daily for morbidity and mortality. Clinical findings and body weights were recorded at the beginning of the study, weekly for 20 weeks, then monthly through week 92, then every 2 weeks until the end of the study.

A complete necropsy and microscopic examination were performed on all animals. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The microscopic 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 ozone studies, a quality assessment pathologist reviewed the lung, nose, and larynx from all animals. In addition, the thyroid was reviewed in male rats, and the clitoral gland was reviewed in female rats.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which a disagreement in diagnosis between the laboratory and quality assessment pathologists existed. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologist, or lesions of general interest were presented by the chair 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. Thus, the final diagnoses represent a consensus of contractor pathologists 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 diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

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 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 as presented in Tables A1, A5, B1, B5, C1, C5, D1, D5, E1, E4, F1, F4, G1, G4, H1, H4, I1, and I4 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, D3, E3, F3, G3, H3, and I3) 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., skin, intestine, harderian gland, and mammary gland) 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.

Analysis of Neoplasm Incidences

The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if the fit of the model was not significantly enhanced. The neoplasm incidences of exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, other methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These methods include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of neoplasm-bearing animals.

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, refer to Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test was used, a procedure based on the overall proportion of affected animals.

Analysis of Continuous Variables

Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). 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' test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's test). Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973).

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, historical control data can be helpful in the overall assessment of neoplasm incidence in certain instances. Consequently, neoplasm incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for neoplasms appearing to show compound-related effects.

Quality Assurance Methods

The 2-year and lifetime 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 and lifetime studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and board draft of this NTP Technical Report were conducted. 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, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of ozone was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*. The protocol for these studies and the results are given in Appendix J.

The genetic toxicity studies of ozone are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure and responses of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests do not correlate well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Ozone and Ozone/NNK

4-Week Studies	2-Year Ozone Studies	Lifetime Ozone Studies	2-Year Ozone/NNK Study
Study Laboratory			
Battelle Pacific Northwest Laboratories (Richland, WA)	Battelle Pacific Northwest Laboratories (Richland, WA)	Battelle Pacific Northwest Laboratories (Richland, WA)	Battelle Pacific Northwest Laboratories (Richland, WA)
Strain and Species			
F344/N rats and $B6C3F_1$ mice	F344/N rats and $B6C3F_1$ mice	F344/N rats and $B6C3F_1$ mice	F344/N rats
Animal Source			
Simonsen Laboratories (Gilroy, CA)	Simonsen Laboratories (Gilroy, CA)	Simonsen Laboratories (Gilroy, CA)	Simonsen Laboratories (Gilroy, CA)
Time Held Before Studies			
13 days	14 days	Rats: 14 days Mice: 21 days	12 days
Average Age When Studies Began			
6 weeks	6 weeks	6 weeks	6 weeks
Date of First Dose			
5 July 1989	Rats: 25 January 1990 Mice: 9 November 1989	Rats: 26 October 1989 Mice: 16 November 1989	Ozone: 28 November 1989 NNK: 27 November 1989
Duration of Dosing			
6 hours (plus T_{90}) per day, 5 days per week, for 4 weeks	6 hours (plus T_{90}) per day, 5 days per week, for 105 weeks	6 hours (plus T_{90}) per day, 5 days per week, for 125 weeks (rats) or 130 weeks (mice)	Ozone: 6 hours (plus T_{90}) per day, 5 days per week, for 105 weeks NNK: in trioctanoin subcutaneously 3 times weekly for 20 weeks
Date of Last Dose			
1 August 1989	Rats: 24 January 1992 Mice: 14 November 1991	Rats: 13 March 1992 Mice: 13 May 1992	Ozone: 27 November 1991 NNK: 13 April 1990
Necropsy Dates			
2 August 1989	Rats: 27-29 January 1992 Mice: 11-15 November 1991	Rats: 17 March 1992 Mice: 14-15 May 1992	2 December 1991
Average Age at Necropsy			
10 weeks	111 weeks	Rats: 131 weeks Mice: 136 weeks	111 weeks

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Ozone and Ozone/NNK (continued)

4-Week Studies	2-Year Ozone Studies	Lifetime Ozone Studies	2-Year Ozone/NNK Study
Size of Study Groups			
Five male and five female rats and mice	50 male and 50 female rats and mice	50 male and 50 female rats and mice	48 male rats
Method of Distribution			
Animals assigned to dose and control groups by a computer generated (XYBION System) table of random numbers. The system used body weight as a blocking variable.	Same as 4-week studies	Same as 4-week studies	Same as 4-week studies
Animals per Cage			
1 per cage compartment	Same as 4-week studies	Same as 4-week studies	Same as 4-week studies
Method of Animal Identification			
Tail tattoo	Same as 4-week studies	Same as 4-week studies	Same as 4-week studies
Diet			
NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, except during exposure periods; changed weekly	Same as 4-week studies	Same as 4-week studies	Same as 4-week studies
Maximum Storage Time for Feed			
120 days post-milling	Same as 4-week studies	Same as 4-week studies	Same as 4-week studies
Water Distribution			
Tap water (Richland municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 4-week studies	Same as 4-week studies	Same as 4-week studies
Cages			
Stainless steel wire bottom cages (Harford Systems, Inc., Aberdeen, MD), cage units rotated in chamber daily	Stainless steel wire bottom cages (Hazleton Systems, Inc., Aberdeen, MD), cage units rotated in chamber weekly	Stainless steel wire bottom cages (Hazleton Systems, Inc., Aberdeen, MD), cage units rotated in chamber weekly	Stainless steel wire bottom cages (Hazleton Systems, Inc., Aberdeen, MD), cage units rotated in chamber weekly

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Ozone and Ozone/NNK (continued)

4-Week Studies	2-Year Ozone Studies	Lifetime Ozone Studies	2-Year Ozone/NNK Study
Chamber Filters			
Single HEPA (Flanders Filters, Inc., San Rafael, CA), and charcoal (RSE, Inc., New Baltimore, MI)	Same as 4-week studies	Same as 4-week studies	Same as 4-week studies
Animal Room Environment			
Average temperature: 23.9° C Relative humidity: 40% to 70% Fluorescent light: 12 hours/day Room air: 12 to 18 changes/hour	Same as 4-week studies	Same as 4-week studies	Same as 4-week studies
Doses			
0, 0.5, or 1.0 ppm	0, 0.12, 0.5, or 1.0 ppm	0, 0.5, or 1.0 ppm	Ozone: 0 or 0.5 ppm NNK: 0, 0.1, or 1.0 mg/kg body weight in trioctanoin, injected subcutaneously
Type and Frequency of Observation			
Observed twice daily; animals were weighed initially, weekly, and at the end of the studies; clinical observations were recorded daily.	Observed twice daily; animals were weighed initially, weekly through week 13, monthly through week 92, then every 2 weeks until the end of the study; clinical observations were recorded initially, monthly through week 92, then every 2 weeks until the end of the study.	Observed twice daily; clinical observations and weights taken initially, monthly through week 91, then every 2 weeks until the end of the study.	Observed twice daily; clinical observations and weights taken initially, weekly for 20 weeks, monthly through week 92, then every 2 weeks until the end of the study.
Method of Sacrifice			
70% CO_2 asphyxiation followed by exsanguination	Same as 4-week studies	Same as 4-week studies	Same as 4-week studies
Necropsy			
Necropsy performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsy performed on all animals.	Necropsy performed on all animals.	Necropsy performed on all animals.

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Ozone and Ozone/NNK (continued)

4-Week Studies	2-Year Ozone Studies	Lifetime Ozone Studies	2-Year Ozone/NNK Study
Histopathology			
Complete histopathology was performed on 0 and 1.0 ppm rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland (rats), esophagus, femur, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lungs, lymph nodes (bronchial and mediastinal), mammary gland (with adjacent skin), muscle (thigh), nose, ovaries, pancreas, parathyroid gland, pituitary gland, preputial gland (rats), prostate gland, salivary gland, spleen, stomach, testes (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, larynx, bronchial and mediastinal lymph nodes, lungs, nose (three sections), thymus, and trachea were examined in 0.5 ppm groups if lesions present in 1.0 ppm group.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland (rats), esophagus, femur, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lungs, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland (with adjacent skin), nose, ovaries, pancreas, parathyroid gland, pituitary gland, preputial gland (rats), prostate gland, salivary gland, spleen, stomach, testes (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland (rats), esophagus, femur, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lungs, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland (with adjacent skin), nose, ovaries, pancreas, parathyroid gland, pituitary gland, preputial gland (rats), prostate gland, salivary gland, spleen, stomach, testes (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.	Histopathology was performed on all animals. In addition to gross lesions and tissue masses, the tissues examined included: lymph nodes (bronchial and mediastinal), lungs, nose, larynx, and trachea.

RESULTS

RATS

4-WEEK STUDY

All rats survived to the end of the study (Table 2). The final mean body weights and mean body weight gains of 0.5 ppm males and females and of 1.0 ppm females were similar to those of the controls. The final mean body weight of 1.0 ppm males was 7% lower than that of the controls.

Clinical findings during the study included hypoactivity and decreased urine and fecal output in 1.0 ppm males and females and ruffled fur in exposed groups of males.

Absolute and relative lung weights of all exposed groups of males and females were greater than those of the controls, and the increases were considered to be related to ozone exposure (Table K1). The absolute lung weight of 1.0 ppm females was significantly greater than that of the controls, as were the relative lung weights of 1.0 ppm males and females. Absolute and relative thymus weights of all exposed groups generally decreased with increasing exposure level, and the absolute and relative thymus weights of 1.0 ppm females were significantly less than those of the controls.

Male and female rats exposed to 0.5 or 1.0 ppm ozone developed patchy, multifocal lesions of the lung involving the centriacinar region; the lesions consisted of infiltration of granulocytes and macrophages with extension of the bronchial epithelium into the alveolar ducts. In addition, exposed groups of males and females developed hyperplasia of the cuboidal nonciliated (transitional) epithelium along the lateral wall of the nasal passage. Female rats exposed to ozone developed minimal squamous metaplasia of the laryngeal epithelium at the base of the epiglottis.

TABLE 2
Survival and Mean Body Weights of Rats in the 4-Week Inhalation Study of Ozone

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	5/5	115 ± 3	242 ± 6	126 ± 5	
0.5	5/5	109 ± 7	238 ± 9	129 ± 7	98
1.0	5/5	112 ± 1	224 ± 5	113 ± 4	93
Female					
0	5/5	97 ± 2	148 ± 1	51 ± 2	
0.5	5/5	100 ± 3	155 ± 5	55 ± 3	105
1.0	5/5	98 ± 2	144 ± 3	46 ± 2	97

[a] Number of animals surviving/number of animals initially in group
[b] Weights and weight changes are given as mean ± standard error.

2-Year Ozone Study

Survival

Estimates of survival probabilities for male and female rats exposed to ozone by inhalation for 2 years are presented in Table 3 and in Kaplan-Meier survival curves (Figure 1). Two-year survival rates of exposed rats were similar to those of the controls.

Body Weights and Clinical Findings

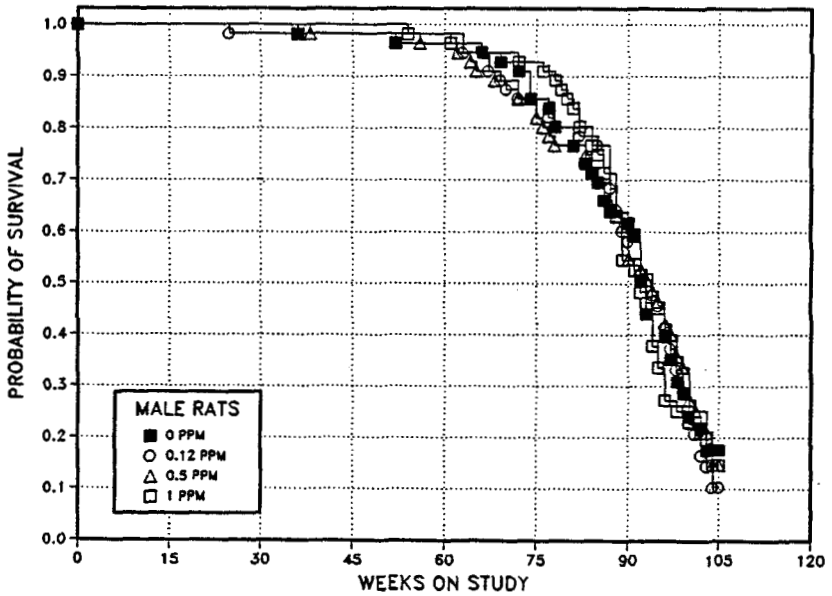
The mean body weights of 0.12 and 0.5 ppm males and females were similar to those of the controls throughout the study, as were the final mean body weights of rats in these exposure groups (Tables 4 and 5 and Figure 2). The mean body weights of 1.0 ppm males and females were slightly lower than those of the controls throughout the study. The final mean body weights of 1.0 ppm males and females were approximately 6% lower than those of the controls.

Hypoactivity was observed in male and female rats exposed to ozone. Rats, particularly those exposed to 1.0 ppm, were less active during and immediately after exposure.

Table 3
Survival of Rats in the 2-Year Inhalation Study of Ozone

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	1			
Moribund	35	40	36	36
Natural deaths	6	5	7	7
Animals surviving to study termination	8	5	7	7
Percent probability of survival at end of study[b]	18	10	15	15
Mean survival (days)[c]	618	620	617	626
Survival analysis[d]	P=0.936	P=0.876	P=1.000N	P=0.870
Female				
Animals initially in study	50	50	50	50
Moribund	19	22	17	16
Natural deaths	3	4	3	7
Animals surviving to study termination	28	24	30	27
Percent probability of survival at end of study	57	50	61	55
Mean survival (days)	668	661	676	648
Survival analysis	P=0.931N	P=0.535	P=0.729N	P=0.866

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A negative trend or a lower mortality in an exposure group is indicated by N.



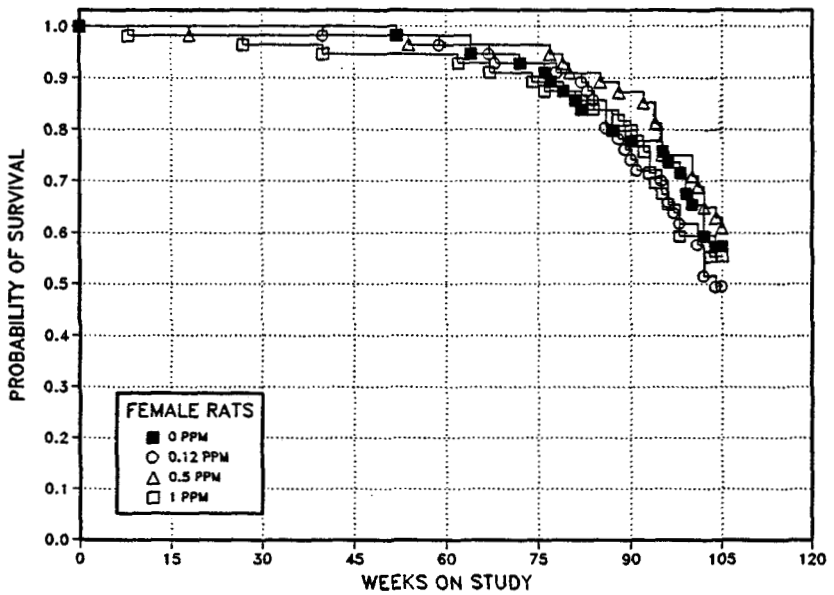


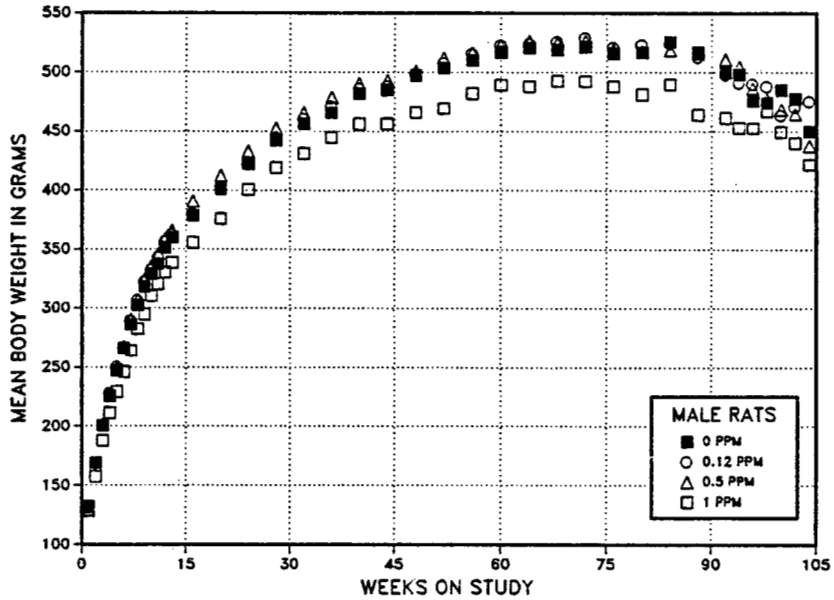
FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Ozone by Inhalation for 2 Years

TABLE 4
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Ozone

Weeks on Study	0 ppm		0.12 ppm			0.5 ppm			1.0 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	132	56	131	99	56	129	97	56	129	98	56
2	169	56	168	99	56	167	99	56	158	93	56
3	201	56	201	100	56	202	101	56	188	94	56
4	225	56	228	101	56	227	101	56	211	94	56
5	247	56	250	101	56	249	101	56	229	93	56
6	266	56	267	100	56	267	101	56	246	92	56
7	286	56	289	101	56	290	101	56	264	92	56
8	302	56	307	102	56	307	102	56	282	93	56
9	318	56	322	101	56	325	102	56	295	93	56
10	329	56	332	101	56	335	102	56	310	94	56
11	337	56	343	102	56	346	103	56	320	95	56
12	351	56	356	101	56	358	102	56	330	94	56
13	360	56	362	101	56	366	102	56	338	94	56
16	378	56	380	100	56	390	103	56	355	94	56
20	401	56	403	101	56	413	103	56	376	94	56
24	423	56	422	100	56	433	102	56	401	95	56
28	442	56	445	101	55	453	102	56	419	95	56
32	456	56	460	101	55	466	102	56	431	95	56
36	466	56	471	101	55	479	103	56	445	96	56
40	482	55	486	101	55	491	102	55	456	95	56
44	485	55	488	101	55	493	102	55	456	94	56
48	497	55	499	100	55	501	101	55	466	94	56
52	504	54	508	101	55	512	102	55	469	93	56
56	510	54	516	101	55	517	101	54	482	95	55
60	517	54	523	101	55	523	101	54	489	95	55
64	521	54	524	101	53	526	101	52	488	94	54
68	519	53	526	101	51	525	101	50	493	95	53
72	522	51	529	102	49	527	101	49	493	94	53
76	516	48	521	101	48	519	101	46	488	95	51
80	517	45	523	101	45	518	100	43	481	93	49
84	526	41	524	100	44	519	99	41	490	93	44
88	517	30	513	99	33	516	100	31	464	90	34
92	500	27	498	100	28	511	102	26	461	92	25
94	498	20	491	99	25	505	101	24	453	91	22
96	476	20	490	103	22	486	102	22	453	95	16
98	474	16	488	103	18	477	101	19	467	98	13
100	485	13	464	96	14	469	97	16	450	93	12
102	478	11	471	99	10	465	97	12	440	92	11
104	450	8	475	106	7	438	97	8	422	94	9
Mean for weeks											
1-13	271		274	101		274	101		254	94	
14-52	453		456	101		463	102		427	94	
53-104	502		505	101		503	100		470	94	

TABLE 5
Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of Ozone

Weeks on Study	0 ppm		0.12 ppm			0.5 ppm			1.0 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	105	56	104	99	56	103	97	56	102	97	56
2	122	56	124	102	56	122	100	56	116	96	56
3	136	56	138	102	56	136	100	56	129	95	56
4	147	56	149	101	56	145	99	56	137	93	56
5	155	56	157	101	56	155	100	56	145	93	56
6	162	56	166	102	56	164	101	56	154	95	56
7	171	56	176	103	56	172	101	56	164	96	56
8	176	56	180	102	56	177	101	56	169	96	55
9	184	56	186	101	56	182	99	56	173	94	55
10	188	56	192	102	56	188	100	56	179	95	55
11	189	56	195	103	56	192	101	56	184	97	55
12	198	56	202	102	56	197	100	56	189	96	55
13	201	56	202	101	56	198	99	56	190	95	55
16	208	56	211	102	56	205	98	56	195	94	55
20	218	56	220	101	56	214	99	55	202	93	55
24	224	56	226	101	56	222	99	55	209	93	55
28	234	56	233	100	56	230	98	55	215	92	54
32	243	56	244	100	56	238	98	55	224	92	54
36	250	56	252	101	56	248	99	55	231	93	54
40	262	56	266	102	56	257	98	55	239	91	53
44	269	56	275	102	55	267	100	55	248	92	53
48	282	56	288	102	55	279	99	55	257	91	53
52	293	56	302	103	55	291	99	55	268	92	53
56	303	55	312	103	55	303	100	54	283	93	53
60	315	55	322	102	54	313	99	54	291	92	53
64	317	55	325	103	54	318	101	54	295	93	52
68	315	53	327	104	52	323	102	54	302	96	51
72	325	53	335	103	52	331	102	54	307	95	51
76	334	52	345	103	52	334	100	54	316	94	49
80	341	49	350	103	51	340	100	52	320	94	49
84	344	47	351	102	49	346	101	51	323	94	48
88	346	39	353	102	39	347	100	44	322	93	41
92	353	38	362	103	35	355	101	42	329	93	38
94	354	38	360	102	35	352	100	42	332	94	35
96	352	37	361	103	34	359	102	37	334	95	33
98	348	36	355	102	31	356	102	37	334	96	32
100	353	33	355	100	30	353	100	37	334	95	29
102	350	32	356	102	27	349	100	34	335	96	29
104	360	29	357	99	25	353	98	32	337	94	27
Mean for weeks											
1-13	164		167	102		164	100		156	95	
14-52	248		252	102		245	99		229	92	
53-104	338		345	102		340	101		318	94	



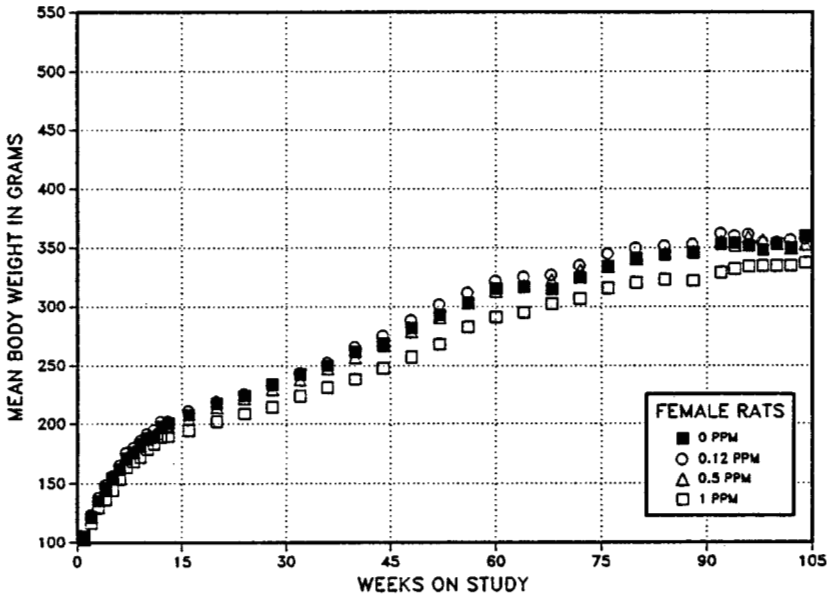


FIGURE 2
Growth Curves for Male and Female Rats Exposed to Ozone by Inhalation for 2 Years

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions in the lung, nose, and larynx. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal respiratory system tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male rats and Appendix B for female rats.

Lung: Males and females exposed to ozone developed increased incidences of metaplasia, inflammation (histiocytic infiltration), and fibrosis (Table 6). The most prominent ozone-related pulmonary lesion was the patchy, multifocal centriacinar extension of cuboidal (ciliated and nonciliated) epithelium into the proximal avleoli and along the alveolar septa. Some of the cuboidal cells appeared to have apical blebs consistent with Clara cells. Because these were uncommon lesions of mild severity in 0.12 ppm rats and were present in nearly all animals exposed to 0.5 or 1.0 ppm, they were clearly ozone concentration dependent. There was an increase in the number of macrophages in the centriacinar alveoli and there was increased thickness (fibrosis) of the adjacent alveolar septa. There was no ozone-related increased incidence of pulmonary neoplasms.

Nose: There were increased incidences of inflammation, hyperplasia, and metaplasia in the nasal passages of rats exposed to ozone (Table 6). Goblet cell hyperplasia was characterized by an increased number of goblet cells within the respiratory epithelium, and hyperplasia of the transitional epithelium was characterized by increased thickness of the cuboidal cell layer. Exposed groups of rats developed flattened and patchy squamous metaplasia of the anterior portion of the transitional epithelium along the lateral wall and on the tips of the maxilloturbinates and nasoturbinates. Increased numbers of lymphocytes and macrophages occurred in the nasal mucosa and increased numbers of granulocytes were observed in the nasal passage. Suppurative inflammation appeared to be more prominent in males than in females.

Larynx: Incidences of metaplasia were observed in the larynx of exposed rats (Table 6), and the lesion was characterized by one or more layers of flattened cells in areas where the epithelium is typically more cuboidal. Ciliated cells which can be occasionally observed were, for the most part, absent in rats exposed to 0.5 or 1.0 ppm ozone.

TABLE 6
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Rats in the 2-Year Inhalation Study of Ozone

Dose (ppm)	0	0.12	0.5	1.0
Male				
Larynx[a]	50	50	50	50
Epiglottis, Metaplasia, Squamous[b]	0	2 (2.5)[c]	16** (1.3)	43** (2.3)
Nose	50	50	50	50
Inflammation, Suppurative	3 (1.7)	10* (1.7)	12* (1.8)	20** (1.9)
Goblet Cell, Lateral Wall, Hyperplasia	1 (2.0)	4 (1.5)	41** (1.5)	48** (2.1)
Lateral Wall, Hyperplasia	0	8** (2.3)	50** (2.0)	49** (2.7)
Lateral Wall, Metaplasia, Squamous	2 (1.5)	6 (1.8)	36** (1.8)	46** (2.3)
Lung	50	50	50	50
Alveolar Epithelium, Metaplasia	0	9** (1.0)	46** (1.9)	47** (2.9)
Alveolus, Infiltration Cellular, Histiocyte	1 (2.0)	0	27** (1.2)	42** (1.9)
Interstitial, Fibrosis	0	2 (1.0)	40** (1.4)	44** (2.2)
Alveolar/bronchiolar Adenoma				
Overall rate[d]	1/50 (2%)	2/50 (4%)	2/50 (4%)	3/50 (6%)
Adjusted rate[e]	2.2%	16.4%	20.4%	25.4%
Terminal rate[f]	0/8 (0%)	0/5 (0%)	1/7 (14%)	1/7 (14%)
First incidence (days)	514	537	698	619
Logistic regression test[g]	P=0.246	P=0.500	P=0.501	P=0.309
Alveolar/bronchiolar Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
Alveolar/bronchiolar Adenoma or Carcinoma[h]				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	4/50 (8%)
Adjusted rate	14.4%	18.6%	33.7%	30.1%
Terminal rate	1/8 (13%)	0/5 (0%)	2/7 (29%)	1/7 (14%)
First incidence (days)	514	537	698	619
Logistic regression test	P=0.284	P=0.500	P=0.515	P=0.341

(continued)

TABLE 6
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Rats in the 2-Year Inhalation Study of Ozone (continued)

Dose (ppm)	0	0.12	0.5	1.0
Female				
Larynx	50	50	50	50
Epiglottis, Metaplasia, Squamous	4 (3.3)	5 (2.8)	9 (2.3)	43** (2.3)
Nose	50	50	50	50
Goblet Cell, Lateral Wall, Hyperplasia	1 (2.0)	2 (1.0)	45** (1.7)	50** (2.5)
Lateral Wall, Hyperplasia	2 (2.0)	8 (1.5)	48** (1.8)	50** (2.6)
Lateral Wall, Metaplasia, Squamous	2 (2.5)	11** (1.4)	21** (1.8)	45** (1.9)
Suppurative Inflammation	3 (1.0)	6 (1.5)	2 (1.0)	2 (2.0)
Lung	50	50	50	50
Alveolar Epithelium, Metaplasia	0	6** (1.0)	48** (1.7)	48** (2.8)
Alveolus, Infiltration Cellular, Histiocyte	0	0	31** (1.2)	43** (1.8)
Interstitial, Fibrosis	0	0	42** (1.4)	47** (2.0)
Alveolar/bronchiolar Adenoma[i]				
Overall rate	0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	6.4%	0.0%
Terminal rate	0/28 (0%)	0/24 (0%)	1/30 (3%)	0/27 (0%)
First incidence (days)	–[j]	–	723	–
Logistic regression test	P=0.545	–	P=0.255	–

* Significantly different (P≤0.05) from the control group by the logistic regression test
** P≤0.01
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals (1=minimal; 2=mild; 3=moderate; 4=marked).
[d] Number of animals with neoplasm per number of animals necropsied
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal sacrifice
[g] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards these lesions as nonfatal.
[h] Historical incidence for 2-year inhalation studies with untreated control groups (mean ± standard deviation): 17/398 (4.3% ± 4.5%); range, 0%-10%
[i] Historical incidence: 4/398 (1.0% ± 1.5%); range, 0%-4%
[j] Not applicable; no neoplasms in animal group

LIFETIME STUDY

Survival

Estimates of survival probabilities for male and female rats exposed to ozone by inhalation for 125 weeks are presented in Table 7 and in Kaplan-Meier survival curves (Figure 3). Survival rates of exposed rats were similar to those of the controls.

Body Weights and Clinical Findings

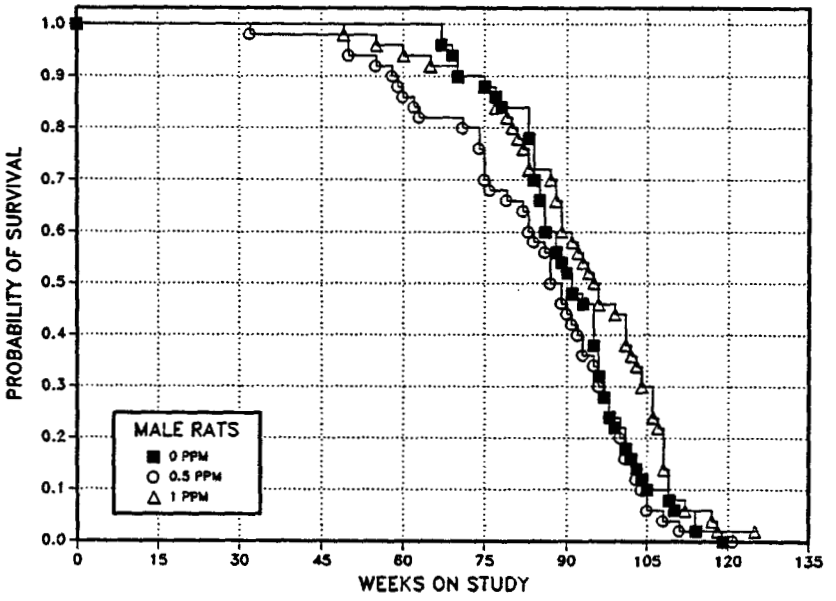
The mean body weights and body weight gains of 1.0 ppm males and females were slightly lower than those of the controls throughout most of the study (Tables 8 and 9 and Figure 4). However, the final mean body weight of all exposed groups were similar to those of the controls.

Hypoactivity was observed in male and female rats exposed to ozone. Rats, particularly those exposed to 1.0 ppm, were less active during and immediately after exposure.

TABLE 7
Survival of Rats in the Lifetime Inhalation Study of Ozone

	0 ppm	0.5 ppm	1.0 ppm
Male			
Animals initially in study	50	50	50
Moribund	47	43	42
Natural deaths	3	7	7
Animals surviving to study termination	0	0	1
Percent probability of survival at end of study[a]	0	0	2
Mean survival (days)[b]	635	592	652
Survival analysis[c]	P=0.122N	P=0.527	P=0.172N
Female			
Animals initially in study	50	50	50
Moribund	36	37	40
Natural deaths	8	7	3
Animals surviving to study termination	6	6	7
Percent probability of survival at end of study	12	12	14
Mean survival (days)	670	726	703
Survival analysis	P=0.402N	P=0.123N	P=0.437N

[a] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A negative trend or a lower mortality in an exposure group is indicated by N.



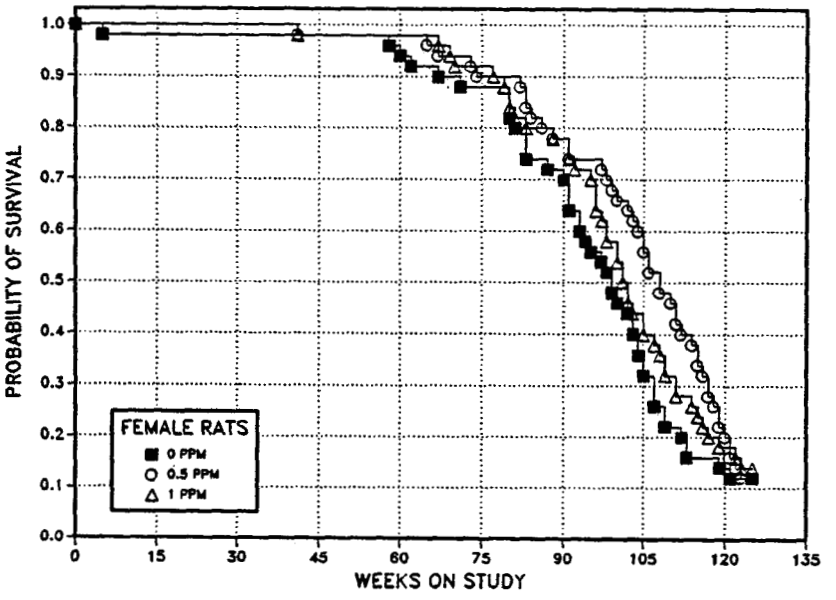


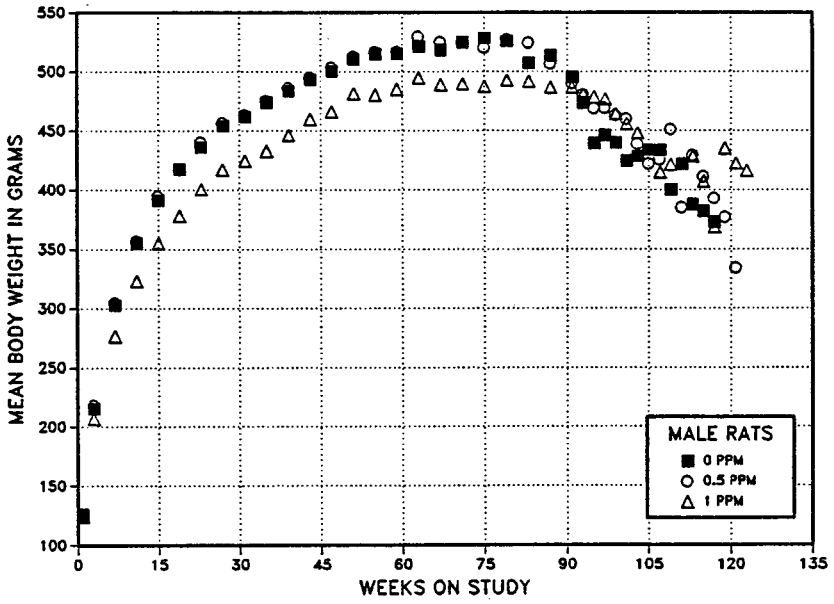
FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Ozone by Inhalation for 124 Weeks

TABLE 8
Mean Body Weights and Survival of Male Rats in the Lifetime Inhalation Study of Ozone

Weeks on Study	0 ppm		0.5 ppm			1.0 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	126	50	125	99	50	125	99	50
3	215	50	219	102	50	207	97	50
7	302	50	305	101	50	278	92	50
11	355	50	357	101	50	325	92	50
15	391	50	396	101	50	357	91	50
19	418	50	418	100	50	380	91	50
23	436	50	441	101	50	402	92	50
27	454	50	457	101	50	418	92	50
31	461	50	464	101	50	426	92	50
35	473	50	476	101	49	434	92	50
39	483	50	486	101	49	448	93	50
43	493	50	495	101	49	461	94	50
47	500	50	504	101	49	467	94	50
51	510	50	513	101	47	483	95	49
55	515	50	517	100	46	482	94	48
59	514	50	517	101	45	487	95	48
63	520	50	530	102	42	495	95	47
67	517	49	525	102	41	491	95	46
71	525	45	525	100	40	491	94	45
75	528	44	519	98	37	488	92	45
79	525	42	527	100	33	495	94	41
83	504	42	524	104	32	493	98	37
87	512	30	506	99	27	487	95	36
91	495	25	490	99	22	487	98	29
93	473	24	480	101	19	481	102	28
95	439	22	469	107	18	479	109	26
97	446	14	469	105	14	477	107	23
99	440	12	463	105	12	464	106	23
101	424	11	460	108	9	456	108	21
103	428	7	439	102	8	448	105	17
105	433	6	422	97	5	434	100	16
107	434	5	425	98	3	415	96	12
109	400	5	451	113	2	421	105	6
111	422	3	385	91	2	422	100	4
113	388	3	429	111	1	428	111	3
115	382	1	411	108	1	407	107	3
117	373	1	393	105	1	368	99	3
119			377		1	435		1
121			334		1	422		1
123						416		1
Mean for weeks								
1-13	250		252	101		234	94	
14-52	462		465	101		428	93	
53-123	462		463	100		457	99	

TABLE 9
Mean Body Weights and Survival of Female Rats in the Lifetime Inhalation Study of Ozone

Weeks on Study	0 ppm		0.5 ppm			1.0 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	103	50	103	99	50	103	100	50
3	145	50	146	101	50	139	96	50
7	180	49	180	100	50	170	95	50
11	202	49	201	100	50	190	94	50
15	215	49	212	99	50	201	94	50
19	229	49	223	97	50	214	93	50
23	236	49	236	100	50	222	94	50
27	246	49	244	99	50	232	94	50
31	256	49	253	99	50	239	93	50
35	267	49	264	99	50	247	93	50
39	278	49	276	99	50	256	92	50
43	293	49	290	99	49	272	93	49
47	304	49	303	100	49	285	94	49
51	313	49	311	100	49	297	95	49
55	317	49	318	100	49	305	96	49
59	324	48	326	101	49	314	97	49
63	333	46	338	101	49	322	97	49
67	337	46	341	101	48	322	96	48
71	346	44	350	101	47	332	96	46
75	349	44	349	100	45	332	95	46
79	348	44	353	102	45	338	97	44
83	357	39	359	101	43	344	96	41
87	358	36	362	101	40	347	97	40
91	360	33	358	100	39	349	97	38
93	357	31	358	100	37	342	96	36
95	356	28	355	100	37	346	97	36
97	349	28	353	101	37	355	102	31
99	347	26	357	103	35	358	103	29
101	347	23	360	104	33	354	102	27
103	349	21	353	101	32	353	101	23
105	359	16	356	99	30	355	99	22
107	354	16	359	101	26	358	101	19
109	353	11	352	100	24	358	101	17
111	343	11	342	100	23	351	103	16
113	329	10	341	104	20	358	109	14
115	345	8	332	96	19	343	100	12
117	335	8	337	101	16	343	102	11
119	338	7	353	105	11	337	100	10
121	330	6	348	106	8	323	98	9
123	328	6	345	105	7	322	98	8
Mean for weeks								
1-13	158		158	100		151	96	
14-52	264		261	99		247	94	
53-123	344		348	101		341	99	



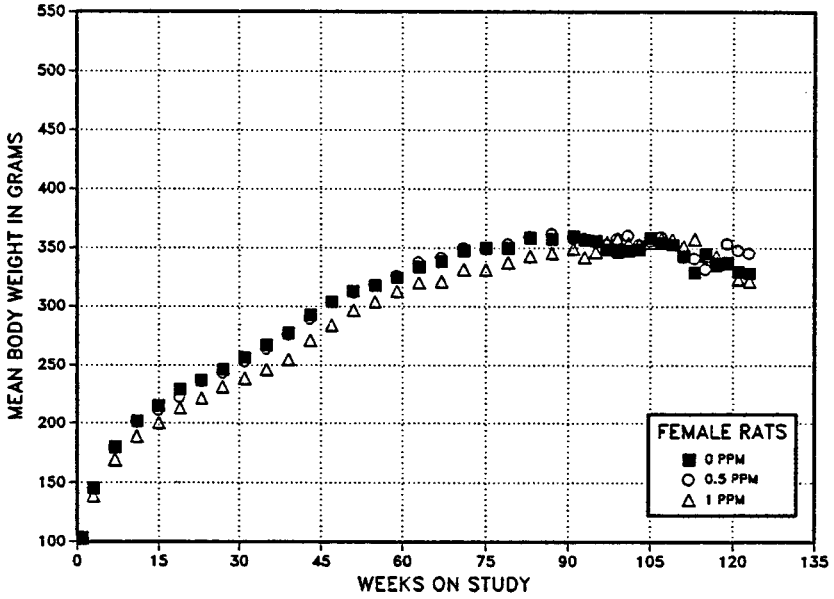


FIGURE 4
Growth Curves for Male and Female Rats Exposed to Ozone by Inhalation for 124 Weeks

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions in the lung, nose, and larynx. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal respiratory system tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, are presented in Appendix F for male rats and Appendix G for female rats.

Lung: Increased incidences of metaplasia, inflammation (histiocytic infiltration), and fibrosis occurred in males and females exposed to 0.5 or 1.0 ppm ozone (Table 10). The ozone-related multifocal centriacinar extension of cuboidal (ciliated and nonciliated) epithelium into the proximal alveoli and along the alveolar septa was similar to that observed in the 2-year study. There was an increase in the number of macrophages in the centriacinar alveoli and increased thickness (fibrosis) of the adjacent alveolar septa. The interstitial fibrosis was more prominent than that observed in the 2-year study. No increased incidences of lung neoplasms were observed.

Nose: Increased incidences of hyperplasia and squamous cell metaplasia were observed in the nasal passages of males and females exposed to 0.5 or 1.0 ppm ozone (Table 10). As in the 2-year study, both goblet cell hyperplasia and hyperplasia of the transitional epithelium were observed. Increased incidences of inflammation were not observed (Tables F4 and G4).

Larynx: Increased incidences of squamous metaplasia occurred in the epiglottis of 0.5 and 1.0 ppm males and females (Table 10). The metaplasia was characterized by one or more layers of flattened cells in areas where the epithelium is typically low cuboidal and appeared similar to that observed in the 2-year studies.

TABLE 10
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Rats in the Lifetime Inhalation Study of Ozone

Dose (ppm)	0	0.5	1.0
Male			
Larynx[a]	50	48	47
Epiglottis, Squamous Metaplasia[b]	0	20** (1.3)[c]	43** (1.8)
Nose	50	49	49
Goblet Cell, Lateral Wall, Hyperplasia	1 (1.0)	46** (1.5)	48** (2.6)
Lateral Wall, Hyperplasia	10 (1.5)	48** (1.9)	47** (2.8)
Lateral Wall, Squamous Metaplasia	10 (2.5)	23** (1.6)	40** (2.3)
Lung	50	50	50
Alveolar Epithelial Metaplasia	0	45** (1.9)	50** (2.9)
Alveolar Cellular Infiltration, Histiocyte	0	38** (1.2)	49** (1.9)
Interstitial Fibrosis	0	44** (1.7)	50** (2.4)
Alveolar/bronchiolar Adenoma			
Overall rate[d]	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate[e]	25.9%	22.3%	0.0%
Terminal rate[f]	0/0	0/0	0/1 (0%)
First incidence (days)	708	581	–[h]
Logistic regression test[g]	P=0.161N	P=0.427	P=0.169N
Alveolar/bronchiolar Carcinoma			
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)
Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rate	25.9%	26.2%	0.0%
Terminal rate	0/0	0/0	0/1 (0%)
First incidence (days)	708	581	–
Logistic regression test	P=0.182N	P=0.266	P=0.169N

(continued)

TABLE 10
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Rats in the Lifetime Inhalation Study of Ozone (continued)

Dose (ppm)	0	0.5	1.0
Female			
Larynx	49	47	50
Epiglottis, Squamous Metaplasia	2 (2.0)	16** (1.1)	48** (2.0)
Nose	50	49	50
Goblet Cell, Lateral Wall, Hyperplasia	0	47** (1.8)	50** (2.4)
Lateral Wall, Hyperplasia	4 (1.8)	49** (1.9)	50** (2.8)
Lateral Wall, Squamous Metaplasia	5 (2.4)	25** (1.3)	35** (1.6)
Lung	50	50	50
Alveolar Epithelial Metaplasia	0	44** (1.7)	50** (2.9)
Alveolar Cellular Infiltration, Histiocyte	0	38** (1.1)	49** (2.0)
Interstitial Fibrosis	0	41** (1.2)	50** (2.5)
Alveolar/bronchiolar Adenoma			
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)
Adjusted rate	0.0%	3.0%	3.3%
Terminal rate	0/6 (0%)	0/6 (0%)	0/7 (0%)
First incidence (days)	–	710	685
Logistic regression test	P=0.330	P=0.507	P=0.500
Alveolar/bronchiolar Carcinoma			
Overall rate	1/50 (2%)	1/50 (2%)	0/50 (0%)
Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	1/50 (2%)	2/50 (4%)	1/50 (2%)
Adjusted rate	12.5%	8.7%	3.3%
Terminal rate	0/6 (0%)	0/6 (0%)	0/7 (0%)
First incidence (days)	827	710	685
Logistic regression test	P=0.594N	P=0.598	P=0.738N

** Significantly different (P≤0.01) than the control group by the logistic regression test
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals (1=minimal; 2=mild; 3=moderate; 4=marked).
[d] Number of animals with neoplasm per number of animals necropsied
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal sacrifice
[g] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards these lesions as nonfatal. A negative trend or a lower incidence in an exposure group is indicated by N.
[h] Not applicable; no neoplasms in animal group

2-Year Ozone/NNK Study

Survival

Estimates of survival probabilities for male rats exposed to ozone and 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or NNK only are presented in Table 11 and in Kaplan-Meier survival curves (Figure 5). Two-year survival rates of all groups of exposed male rats were similar.

Body Weights and Clinical Findings

Final mean body weights of all males exposed to NNK alone or NNK and ozone were similar to that of the controls, with the exception of rats exposed to 1.0 mg NNK/kg body weight and 0.5 ppm ozone. The mean body weights of exposed and control groups were similar throughout the study (Table 12 and Figure 6).

Hypoactivity was observed in males exposed to NNK and ozone and in those exposed to NNK without ozone. Rats were less active during and immediately after exposure.

Table 11
Survival of Male Rats in the 2-Year Inhalation Study of Ozone/NNK

	Vehicle/ 0 ppm Ozone	Vehicle/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Animals initially in study	48	48	48	48	48	48
Moribund	36	36	40	38	41	36
Natural deaths	4	9	2	4	3	7
Animals surviving to study termination	8	3	6	6	4	5
Percent probability of survival at end of study[a]	17	6	13	13	8	10
Mean survival days[b]	638	595	618	622	617	622
Survival analysis[c]		P=0.094	P=0.370	P=0.666	P=0.122	P=0.394

[a] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The results of the life table pairwise comparisons (Cox, 1972) with the control are in the exposed column.

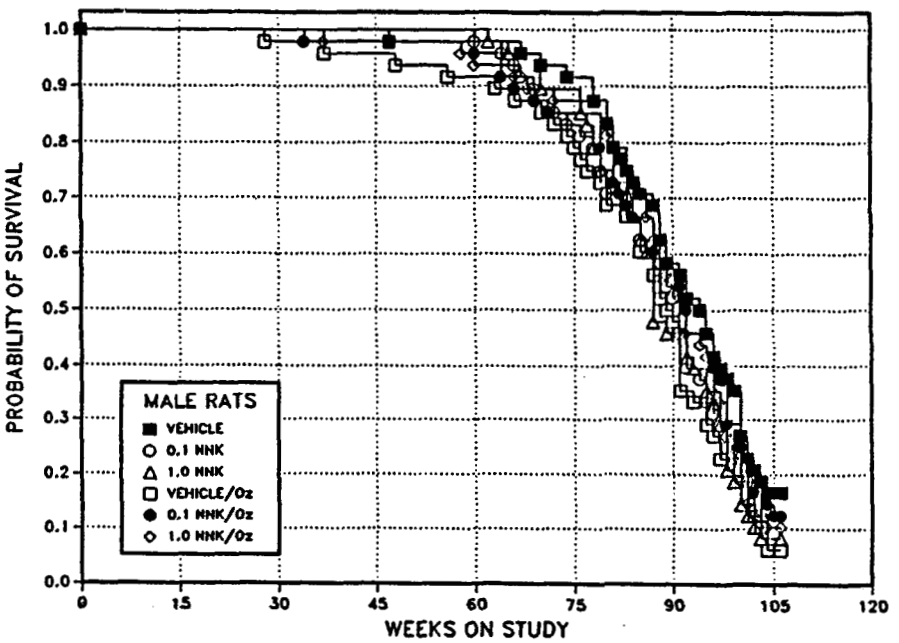


FIGURE 5
Kaplan-Meier Survival Curves for Male Rats Exposed to Ozone or Ozone/NNK by Inhalation for 2 Years

TABLE 12
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Ozone and Ozone/NNK

Weeks on Study	Vehicle Control		0.1 mg/kg NNK/ 0 ppm Ozone			1.0 mg/kg NNK/ 0 ppm Ozone			Vehicle Control/ 0.5 ppm Ozone		
	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	114	48	114	100	48	114	100	48	113	99	48
2	156	48	156	99	48	155	99	48	157	100	48
3	192	48	193	101	48	190	99	48	192	100	48
4	221	48	223	101	48	221	100	48	223	101	48
5	244	48	246	101	48	244	100	48	248	102	48
6	263	48	265	101	48	264	100	48	269	102	48
7	277	48	282	102	48	282	102	48	285	103	48
8	293	48	300	103	48	299	102	48	302	103	48
9	308	48	315	102	48	314	102	48	316	102	48
10	320	48	327	102	48	326	102	48	331	103	48
11	329	48	340	103	48	338	103	48	339	103	48
12	340	48	348	102	48	349	102	48	350	103	48
13	349	48	357	102	48	359	103	48	359	103	48
14	358	48	367	103	48	368	103	48	370	104	48
15	368	48	377	103	48	378	103	48	379	103	48
16	376	48	384	102	48	385	102	48	388	103	48
17	383	48	391	102	48	391	102	48	394	103	48
18	391	48	400	102	48	402	103	48	403	103	48
19	396	48	408	103	48	410	104	48	408	103	48
20	404	48	415	103	48	419	104	48	414	102	48
24	421	48	427	101	48	431	102	48	426	101	48
28	427	48	441	103	48	442	103	48	436	102	47
32	442	48	453	103	48	454	103	48	452	102	47
36	455	48	467	103	48	468	103	48	464	102	47
40	468	48	481	103	48	480	102	48	479	102	46
44	478	48	493	103	48	490	103	48	489	102	46
48	485	47	500	103	48	499	103	48	494	102	45
52	489	47	501	103	48	499	102	48	498	102	45
56	494	47	506	102	48	506	102	48	504	102	45
60	503	47	515	102	48	513	102	48	508	101	44
64	506	47	521	103	46	517	102	47	509	101	43
68	507	46	521	103	44	515	101	46	509	100	42
72	507	45	519	102	41	516	102	43	504	99	41
76	515	44	528	103	39	522	102	41	516	100	37
80	507	40	522	103	35	520	103	36	513	101	34
84	499	36	517	104	32	501	100	32	508	102	32
88	500	31	503	101	29	500	100	23	502	100	26
92	498	26	515	103	21	484	97	21	500	100	17
94	487	25	518	107	19	475	98	19	495	102	16
96	474	22	512	108	16	461	97	17	478	101	14
98	483	18	488	101	14	449	93	14	486	101	11
100	463	17	479	103	12	472	102	7	466	101	10
102	471	10	499	104	8	447	95	6	458	97	6
104	469	8	499	107	6	474	101	4	463	99	4
Mean for weeks											
1-13	262		267	102		266	102		268	102	
14-52	423		434	103		434	103		433	102	
53-104	493		510	103		492	100		495	100	

(continued)

TABLE 12
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Ozone and Ozone/NNK
(continued)

Weeks on Study	Vehicle Control		Vehicle Control/ 0.5 ppm Ozone			0.1 mg/kg NNK/ 0.5 ppm Ozone			1.0 mg/kg NNK/ 0.5 ppm Ozone		
	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	114	48	113	99	48	111	97	48	114	99	48
2	157	48	157	100	48	154	98	48	157	100	48
3	192	48	192	100	48	189	99	48	188	98	48
4	221	48	223	101	48	219	99	48	220	100	48
5	244	48	248	102	48	241	99	48	243	100	48
6	263	48	269	102	48	261	99	48	265	101	48
7	277	48	285	103	48	278	100	48	282	102	48
8	293	48	302	103	48	292	100	48	299	102	48
9	308	48	316	102	48	307	100	48	313	102	48
10	320	48	331	103	48	323	101	48	328	103	48
11	329	48	339	103	48	335	102	48	341	104	48
12	340	48	350	103	48	345	101	48	351	103	48
13	349	48	359	103	48	354	102	48	360	103	48
14	358	48	370	104	48	364	102	48	371	104	48
15	368	48	379	103	48	374	102	48	380	103	48
16	376	48	388	103	48	380	101	48	387	103	48
17	383	48	394	103	48	385	101	48	394	103	48
18	391	48	403	103	48	396	101	48	404	103	48
19	396	48	408	103	48	402	102	48	412	104	48
20	404	48	414	102	48	410	102	48	417	103	48
24	421	48	426	101	48	419	100	48	428	102	48
28	427	48	436	102	47	432	101	48	434	102	48
32	442	48	452	102	47	448	101	48	455	103	48
36	455	48	464	102	47	461	101	47	467	103	48
40	468	48	479	102	46	475	102	47	483	103	47
44	478	48	489	102	46	485	102	47	493	103	47
48	485	47	494	102	45	493	102	47	501	104	47
52	489	47	498	102	45	498	102	47	506	104	47
56	494	47	504	102	45	500	101	47	509	103	47
60	503	47	508	101	44	505	100	46	516	103	45
64	506	47	509	101	43	511	101	44	517	102	45
68	507	46	509	100	42	508	100	43	521	103	43
72	507	45	504	99	41	502	99	41	511	101	43
76	515	44	516	100	37	518	101	41	521	101	42
80	507	40	513	101	34	512	101	38	511	101	41
84	499	36	508	102	32	511	102	32	512	103	34
88	500	31	502	100	26	514	103	29	512	102	29
92	498	26	500	100	17	498	100	25	497	100	23
94	487	25	495	102	16	491	101	24	483	99	22
96	474	22	478	101	14	479	101	22	464	98	20
98	483	18	486	101	11	473	98	17	450	93	16
100	463	17	466	101	10	470	102	14	436	94	11
102	471	10	458	97	6	471	100	10	439	93	7
104	469	8	463	99	4	473	101	8	431	92	6
Mean for weeks											
1-13	262		268	102		262	100		266	102	
14-52	423		433	102		428	101		435	103	
53-104	492		495	101		496	101		489	99	

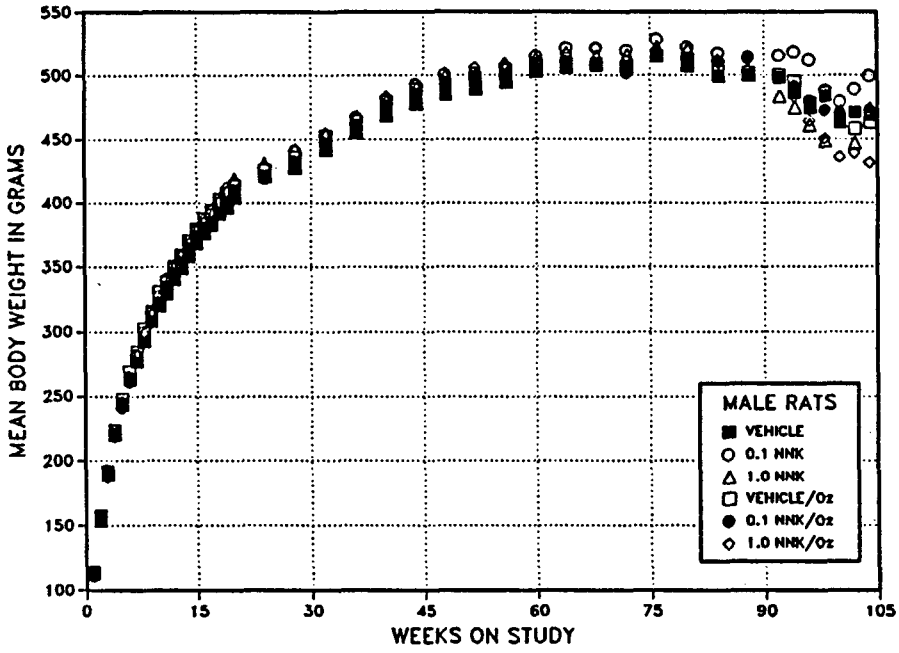


FIGURE 6
Growth Curves for Male Rats Exposed to Ozone or Ozone/NNK by Inhalation for 2 Years

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions in the lung and nose. Summaries of the incidences of nonneoplastic lesions, individual animal respiratory system tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix E.

Lung: Alveolar epithelial metaplasia and interstitial fibrosis occurred in all groups of rats exposed to ozone (with or without NNK), but were not observed in vehicle controls or in animals exposed to NNK alone (Table 13). The incidence of alveolar cellular infiltration was greater in males exposed to ozone than in the vehicle control males. There was a dose-related increased incidence of atypical alveolar hyperplasia in groups of rats receiving NNK, and the increase was significant. An increased incidence of alveolar/bronchiolar adenoma or carcinoma (combined) also occurred in rats administered 1.0 mg/kg NNK, with or without ozone. The administration of ozone did not affect the occurrence of pulmonary neoplasms or nonneoplastic lesions in rats administered NNK.

Nose: The incidence of hyperplasia in groups of rats exposed to ozone with and without NNK was greater than the incidence in males not exposed to ozone. Incidences of hyperplasia among groups of rats exposed only to NNK were low and similar to that of the controls (Table 13). The nasal lesions were similar to those seen in rats exposed to ozone by inhalation for 2 years.

TABLE 13
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone/NNK

(Dose)	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Nose[a]	47	48	48	48	48	46
Goblet Cell, Lateral Wall, Hyperplasia[b]	3 (1.0)[c]	38** (1.0)	0	45** (1.1)	3 (1.3)	42**(1.0)
Lateral Wall, Hyperplasia	5 (1.2)	46** (1.0)	4 (1.5)	48** (1.0)	5 (1.8)	46**(1.0)
Olfactory Epithelial, Hyaline Degeneration	47 (1.1)	47 (2.1)	48 (1.2)	48 (1.9)	45 (1.2)	46 (1.9)
Lung	48	48	48	48	48	48
Alveolar Epithelial Hyperplasia, Atypical	0	0	10** (1.8)	12** (1.7)	39** (2.1)	33**(2.1)
Alveolar Epithelium, Metaplasia	0	35** (1.0)	0	47** (1.0)	0	45**(1.0)
Alveolar Cellular Infiltration, Histiocyte	1 (3.0)	7* (1.1)	1 (2.0)	9** (1.1)	8* (2.3)	13**(1.6)
Interstitial Fibrosis	0	34** (1.1)	0	46** (1.0)	0	45**(1.0)
Alveolar/bronchiolar Adenoma or Carcinoma						
Overall rate[d]	3/48 (6%)	1/48 (2%)	2/48 (4%)	3/48 (6%)	23/48 (48%)	28/48 (58%)
Adjusted rate[e]	37.5%	3.2%	7.7%	35.1%	93.2%	100.0%
Terminal rate[f]	3/8 (38%)	0/3 (0%)	0/6 (0%)	2/6 (33%)	3/4 (75%)	5/5 (100%)
First incidence (days)	736 (T)	590	625	565	429	557
Logistic regression[g]		P=0.442N	P=0.591N	P=0.627	P<0.001	P<0.001

* Significantly different (P≤0.05) than the control group by the logistic regression test
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in all animals (1=minimal; 2=mild; 3=moderate; 4=marked).
[d] Number of animals with neoplasm per number of animals necropsied
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal sacrifice
[g] Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that exposed group. The logistic regression test regards these lesions as nonfatal. A lower incidence in an exposure group is indicated by N.

MICE

4-WEEK STUDY

All mice survived to the end of the study (Table 14). The final mean body weights and body weight gains of all exposed groups of mice were less than those of the controls. Hypoactivity was observed in 1.0 ppm mice throughout the study.

The relative lung weight of 1.0 ppm males was significantly greater than that of the controls (Table K2). There were no other statistically significant differences in absolute or relative organ weights in males or females.

Male and female mice exposed to 0.5 or 1.0 ppm ozone developed patchy, multifocal lesions of the lung involving the centriacinar region; the lesions consisted of infiltration of granulocytes and macrophages with extension of the bronchial epithelium into the alveolar ducts. Slight hyperplasia of ciliated and nonciliated cells was observed in the cuboidal epithelium of the alveolar ducts with a minimal histiocytic infiltrate. In addition, exposed groups of males and females developed hyperplasia of the cuboidal nonciliated (transitional) epithelium along the lateral wall of the nasal passage with an increased number of neutrophils in the epithelial mucosa. No nonneoplastic lesions were observed in the larynx of mice.

TABLE 14
Survival and Body Weights of Mice in the 4-Week Inhalation Study of Ozone

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	5/5	23.6 ± 0.4	31.5 ± 1.2	7.9 ± 1.2	
0.5	5/5	23.4 ± 0.2	29.1 ± 0.7	5.7 ± 0.5	92
1.0	5/5	23.6 ± 0.5	28.9 ± 0.4	5.3 ± 0.5	92
Female					
0	5/5	19.0 ± 0.3	26.7 ± 1.9	7.7 ± 1.9	
0.5	5/5	19.2 ± 0.5	24.3 ± 0.3	5.1 ± 0.6	91
1.0	5/5	19.0 ± 0.5	25.8 ± 1.4	6.8 ± 1.1	97

[a] Number of animals surviving/number of animals initially in group
[b] Weights and weight changes are given as mean ± standard error.

2-Year Study

Survival

Estimates of survival probabilities for male and female mice exposed to ozone by inhalation for 2 years are presented in Table 15 and in Kaplan-Meier survival curves (Figure 7). Two-year survival rates of exposed groups of males and 0.12 and 0.5 ppm females were similar to that of the controls; 2-year survival rates of 1.0 ppm females were marginally greater than that of the controls.

Body Weights and Clinical Findings

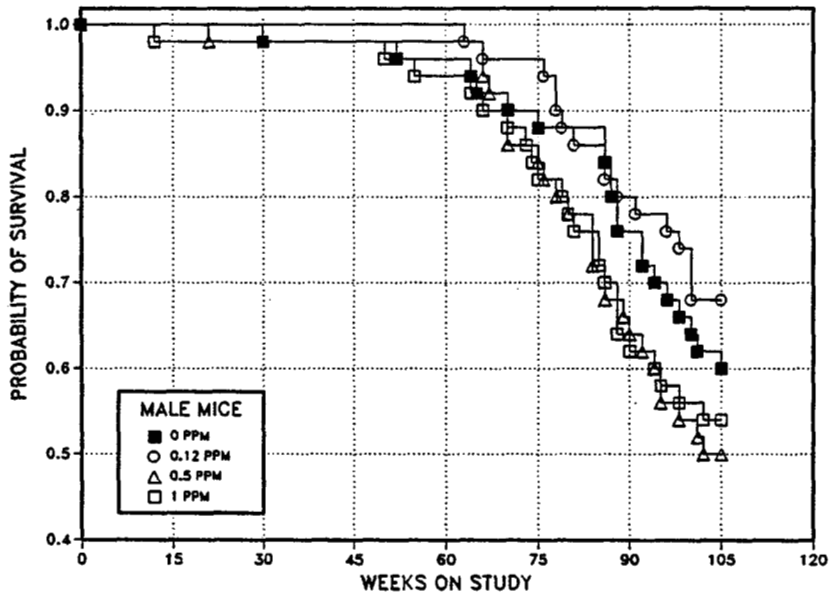
The mean body weights of 0.12 and 0.5 ppm males were similar to those of the controls (Tables 16 and 17 and Figure 8). Mean body weights of 1.0 ppm males and of all exposed groups of females were less than those of the controls.

Hypoactivity was observed in male and female mice exposed to ozone. Mice, particularly those exposed to 1.0 ppm, were less active during and immediately after exposure.

Table 15
Survival of Mice in the 2-Year Inhalation Study of Ozone

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	16	10	19	20
Natural deaths	4	6	6	3
Animals surviving to study termination	30[a]	34	25	27
Percent probability of survival at end of study[b]	60	68	50	54
Mean survival (days)[c]	670	689	647	644
Survival analysis[d]	P=0.157	P=0.519N	P=0.329	P=0.515
Female				
Animals initially in study	50	50	50	50
Accidental deaths[e]			2	
Moribund	15	10	9	9
Natural deaths	6	3	6	1
Animals surviving to study termination	29	37	33	40
Percent probability of survival at end of study	58	74	69	80
Mean survival (days)	691	707	697	703
Survival analysis	P=0.089N	P=0.134N	P=0.276N	P=0.045N

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



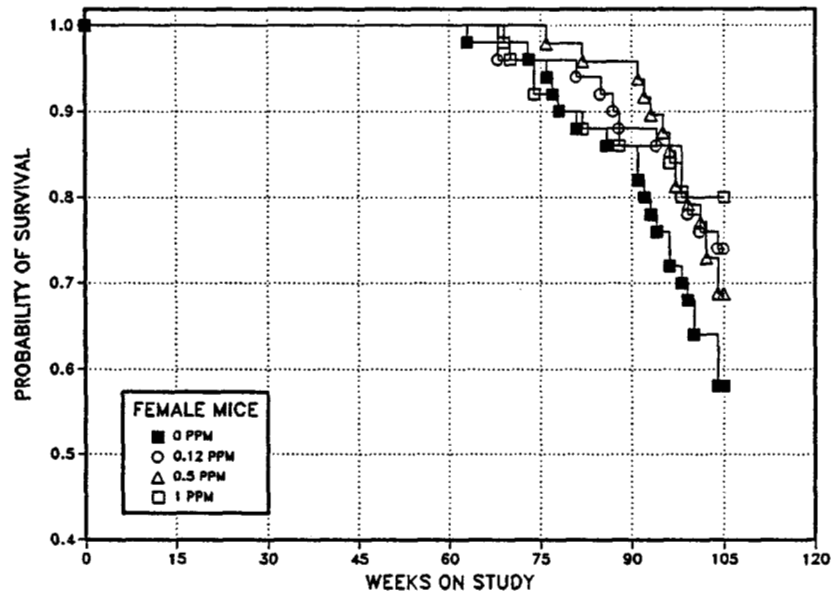


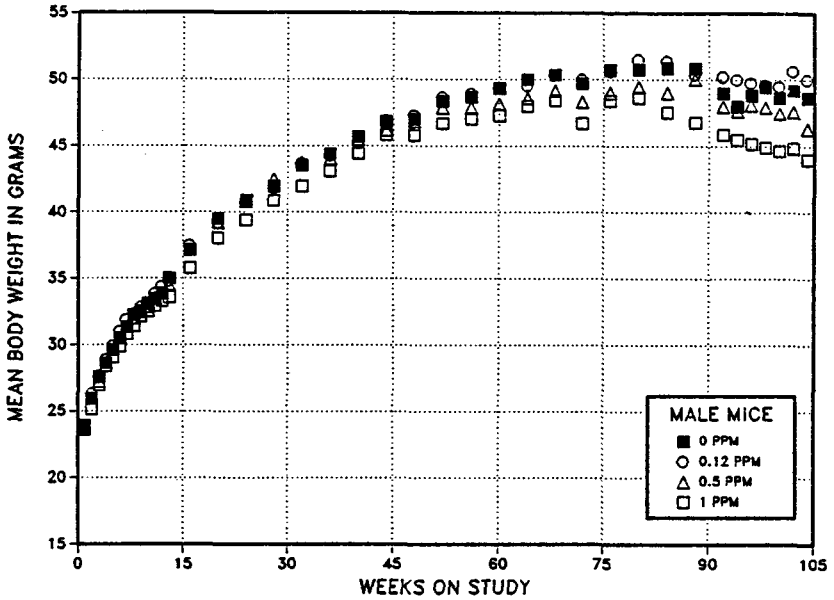
FIGURE 7
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Ozone by Inhalation for 2 Years

TABLE 16
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of Ozone

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	0.12 ppm Av. Wt. (g)	0.12 ppm Wt. (% of controls)	0.12 ppm No. of Survivors	0.5 ppm Av. Wt. (g)	0.5 ppm Wt. (% of controls)	0.5 ppm No. of Survivors	1.0 ppm Av. Wt. (g)	1.0 ppm Wt. (% of controls)	1.0 ppm No. of Survivors
1	23.9	50	23.8	100	50	23.8	100	50	23.6	99	50
2	25.9	50	26.3	102	50	26.0	100	50	25.1	97	50
3	27.6	50	27.6	100	50	27.2	99	50	26.9	98	50
4	28.6	50	28.9	101	50	28.6	100	50	28.4	99	50
5	29.6	50	29.9	101	50	29.7	100	50	29.0	98	50
6	30.5	50	31.0	102	50	30.5	100	50	29.9	98	50
7	31.3	50	31.9	102	50	31.4	100	50	30.8	98	50
8	32.2	50	32.3	100	50	32.0	99	50	31.4	98	50
9	32.5	50	32.8	101	50	32.4	100	50	32.1	99	50
10	33.1	50	33.0	100	50	32.8	99	50	32.5	98	50
11	33.4	50	33.8	101	50	33.5	100	50	32.9	99	50
12	33.9	50	34.4	102	50	33.9	100	50	33.3	98	50
13	35.0	50	34.8	99	50	34.5	99	50	33.5	96	49
16	37.1	50	37.5	101	50	37.2	100	50	35.8	97	49
20	39.5	50	39.2	99	50	39.2	99	50	38.0	96	49
24	40.9	50	40.8	100	50	40.8	100	49	39.4	96	49
28	42.0	50	41.8	100	50	42.5	101	49	40.9	97	49
32	43.5	49	43.7	101	50	43.8	101	49	42.0	97	49
36	44.4	49	44.2	100	50	44.1	99	49	43.1	97	49
40	45.7	49	45.2	99	50	45.5	100	49	44.4	97	49
44	46.8	49	46.9	100	50	46.2	99	49	45.8	98	49
48	47.0	49	47.3	101	50	46.9	100	49	45.8	97	49
52	48.4	49	48.7	101	50	47.9	99	49	46.7	97	48
56	48.7	48	48.9	100	50	47.9	98	49	47.1	97	47
60	49.4	48	49.4	100	50	48.2	98	49	47.3	96	47
64	50.0	48	49.5	99	49	48.6	97	49	48.0	96	47
68	50.3	46	50.3	100	48	49.2	98	46	48.4	96	45
72	49.7	45	50.0	101	48	48.3	97	43	46.7	94	44
76	50.7	44	50.6	100	48	49.0	97	42	48.4	96	41
80	50.8	44	51.5	101	44	49.5	97	40	48.7	96	40
84	50.9	44	51.4	101	43	49.0	96	39	47.6	94	38
88	50.9	40	50.4	99	41	50.0	98	34	46.8	92	35
92	49.0	38	50.2	102	39	48.0	98	32	45.9	94	31
94	48.0	36	50.0	104	39	47.6	99	31	45.5	95	31
96	48.8	35	49.8	102	39	48.1	99	28	45.2	93	29
98	49.5	34	49.6	100	38	48.0	97	28	44.9	91	29
100	48.7	33	49.5	102	37	47.5	98	27	44.7	92	28
102	49.2	31	50.6	103	34	47.6	97	26	44.9	91	28
104	48.7	31	50.0	103	34	46.3	95	25	44.0	90	27
Mean for weeks											
1-13	30.6		30.8	101		30.5	100		30.0	98	
14-52	43.5		43.5	100		43.4	100		42.2	97	
53-104	49.6		50.1	101		48.3	97		46.5	94	

TABLE 17
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Ozone

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	0.12 ppm Av. Wt. (g)	0.12 ppm Wt. (% of controls)	0.12 ppm No. of Survivors	0.5 ppm Av. Wt. (g)	0.5 ppm Wt. (% of controls)	0.5 ppm No. of Survivors	1.0 ppm Av. Wt. (g)	1.0 ppm Wt. (% of controls)	1.0 ppm No. of Survivors
1	19.9	50	20.2	102	50	19.9	100	50	19.9	100	50
2	21.5	50	21.5	100	50	21.4	100	50	20.5	95	50
3	23.2	50	23.0	99	50	22.6	97	50	22.3	96	50
4	24.2	50	24.4	101	50	24.0	99	50	23.5	97	50
5	25.0	50	24.6	98	50	25.0	100	50	24.3	97	50
6	26.3	50	25.7	98	50	26.0	99	50	25.2	96	50
7	27.4	50	27.0	99	50	27.0	99	50	26.1	95	50
8	27.8	50	27.5	99	50	27.5	99	50	26.7	96	50
9	27.7	50	27.5	99	50	28.2	102	50	27.3	99	50
10	28.2	50	27.5	98	50	28.9	103	50	28.0	99	50
11	28.7	50	28.2	98	50	29.5	103	50	28.1	98	50
12	29.4	50	28.7	98	50	30.2	103	50	28.4	97	50
13	29.9	50	29.1	97	50	31.2	104	50	29.1	97	50
16	32.2	50	30.6	95	50	33.2	103	50	31.1	97	50
20	35.0	50	32.5	93	50	36.0	103	50	33.7	96	50
24	36.1	50	34.4	95	50	38.0	105	49	35.1	97	50
28	37.9	50	35.6	94	50	39.8	105	49	36.4	96	50
32	39.7	50	36.0	91	50	40.9	103	49	37.3	94	50
36	41.1	50	37.1	90	50	41.9	102	49	37.1	90	50
40	42.7	50	39.0	91	50	43.6	102	49	38.8	91	50
44	44.7	50	41.0	92	50	44.8	100	49	40.0	90	50
48	45.5	50	42.2	93	50	44.9	99	49	39.9	88	50
52	47.5	50	42.4	89	50	47.2	99	49	41.3	87	50
56	49.0	50	44.3	90	50	47.5	97	49	42.1	86	50
60	49.1	50	45.1	92	50	47.5	97	49	42.7	87	50
64	52.3	49	46.3	89	50	48.6	93	49	43.6	83	50
68	51.6	49	47.1	91	48	49.6	96	49	45.0	87	50
72	51.8	49	47.2	91	48	48.3	93	48	43.7	84	48
76	53.0	48	47.8	90	48	50.5	95	48	46.1	87	46
80	54.2	45	49.6	92	48	51.3	95	47	47.1	87	45
84	54.1	44	49.6	92	47	51.1	95	46	46.3	86	44
88	53.0	43	48.6	92	45	51.4	97	46	47.0	89	44
92	52.6	40	48.2	92	44	49.9	95	45	46.5	88	43
94	51.0	39	47.4	93	44	49.0	96	43	45.6	89	43
96	51.2	37	47.6	93	43	48.5	95	42	45.6	89	43
98	51.7	36	47.1	91	43	48.6	94	39	46.2	89	42
100	51.7	33	47.5	92	39	48.2	93	38	46.8	91	40
102	52.5	32	47.8	91	38	47.6	91	37	47.4	90	40
104	51.4	32	47.8	93	38	46.1	90	35	46.3	90	40
Mean for weeks											
1-13	26.1		25.8	99		26.3	101		25.3	97	
14-52	40.2		37.1	92		41.0	102		37.1	93	
53-104	51.9		47.4	92		49.0	95		45.5	88	



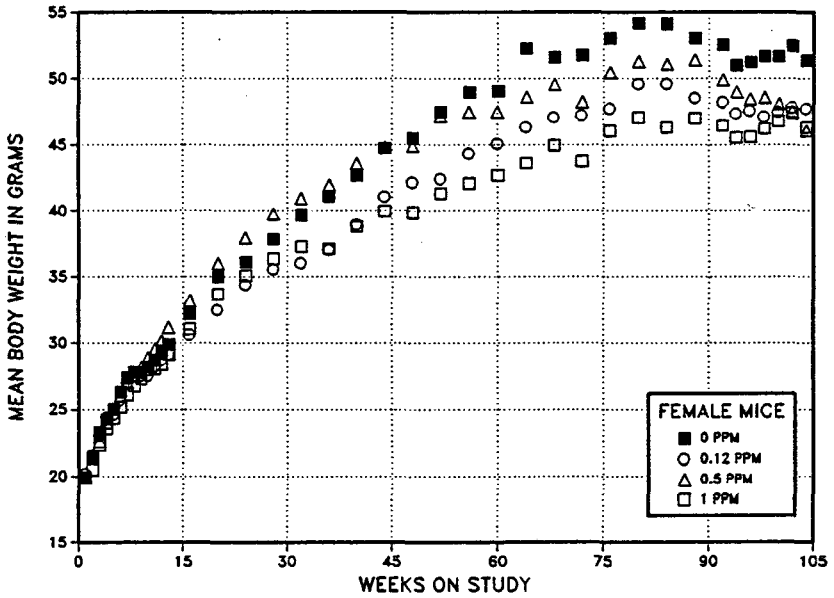


FIGURE 8
Growth Curves for Male and Female Mice Exposed to Ozone by Inhalation for 2 Years

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions in the lung, nose, larynx, and liver. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal respiratory system tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidence data are presented in Appendix C for male mice and Appendix D for female mice.

Lung: The incidence of alveolar/bronchiolar neoplasms increased with increasing ozone exposure. The most prominent increased incidence was that of carcinomas in females. There was also an increase in the number of male mice with multiple adenomas (0 ppm, 0/50; 0.12 ppm, 0/50; 0.5 ppm, 3/50; 1.0 ppm, 1/50; Table C1). The incidence of multiple carcinomas in exposed males was similar to that in controls (2/50, 2/50, 4/50, 2/50; Table C1). One multiple adenoma occurred in a 1.0 ppm female (Table D1). The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) occurred with a significant positive trend, and the incidence in 1.0 ppm females was significantly increased (Table 18). In addition, the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in 0.5 and 1.0 ppm females exceeded the NTP historical control range for this neoplasm (58/659; range, 0%-15%; Table D4).

Slight increased incidences of metaplasia occurred in the cuboidal (ciliated and nonciliated) epithelium in the alveolar ducts, with a minimal histiocytic infiltrate. These lesions were observed in 0.5 and 1.0 ppm males and females. There were no increased incidences of hyperplasia.

Nose: Increased incidences of degeneration, fibrosis, hyperplasia, and squamous metaplasia occurred in 0.5 and 1.0 ppm males and females. Degeneration was also observed in 0.12 ppm females, and increased incidences of inflammation occurred in all exposed groups of males and females (Table 18). The degeneration (hyaline) was characterized by brightly eosinophilic globules of varying sizes in the cytoplasm of epithelial cells lining the nasal passage. This eosinophilic material often filled and distorted the cells. Fibrosis was characterized by increased numbers of fibroblasts and collagen in the mucosa. Hyperplastic epithelium often involved the transitional epithelium along the lateral wall with an increase in the number of cell layers. Patchy areas were observed where the cuboidal epithelium was replaced by squamous epithelium.

Larynx: Increased incidences of hyperplasia occurred in the epiglottis of six males and seven females exposed to 1.0 ppm ozone (Table 18). The hyperplasia consisted of increased numbers of cell layers; the cells tended to be cuboidal with enlarged nuclei.

Liver: There was a decreased incidence of hepatocellular adenoma or carcinoma (combined) in exposed groups of females (0 ppm, 27/50; 0.12 ppm, 22/50; 0.5 ppm, 20/50; 1 ppm, 11/50; Table D3). This decrease did not occur in males in the 2-year study or in male or female mice in the lifetime study (Tables C3, H3, and I3).

TABLE 18
Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory Tract in Mice in the 2-Year Inhalation Study of Ozone

Dose (ppm)	0	0.12	0.5	1.0
Male				
Larynx[a]	50	50	50	50
Epiglottis, Hyperplasia[b]	1 (1.0)[c]	0	0	6 (1.0)
Nose	50	50	50	50
Lateral Wall, Hyaline Degeneration	2 (1.0)	1 (2.0)	49** (2.0)	50** (3.7)
Lateral Wall, Fibrosis	0	0	47** (1.6)	49** (2.7)
Lateral Wall, Hyperplasia	0	0	42** (1.6)	50** (2.3)
Lateral Wall, Inflammation, Suppurative	0	8**(1.0)	42** (1.5)	50** (2.1)
Lateral Wall, Metaplasia, Squamous	0	3 (1.7)	3 (1.0)	36** (1.7)
Lung	50	50	50	50
Alveolar Epithelium, Metaplasia	0	0	48** (1.6)	50** (2.6)
Alveolus, Infiltration Cellular, Histiocyte	0	0	18** (1.1)	31** (1.8)
Alveolar Epithelium, Hyperplasia	4 (1.5)	6 (2.3)	2 (2.0)	3 (3.3)
Alveolar/bronchiolar Adenoma				
Overall rate[d]	6/50 (12%)	9/50 (18%)	12/50 (24%)	11/50 (22%)
Adjusted rate[e]	18.8%	25.1%	40.9%	34.7%
Terminal rate[f]	5/30 (17%)	8/34 (24%)	9/25 (36%)	8/27 (30%)
First incidence (days)	611	440	464	484
Logistic regression test[g]	P=0.079	P=0.318	P=0.061	P=0.110
Alveolar/bronchiolar Carcinoma				
Overall rate	8/50 (16%)	4/50 (8%)	8/50 (16%)	10/50 (20%)
Adjusted rate	25.5%	10.3%	30.7%	35.4%
Terminal rate	7/30 (23%)	1/34 (3%)	7/25 (28%)	9/27 (33%)
First incidence (days)	653	612	701	630
Logistic regression test	P=0.062	P=0.154N	P=0.449	P=0.270
Alveolar/bronchiolar Adenoma or Carcinoma[h]				
Overall rate	14/50 (28%)	13/50 (26%)	18/50 (36%)	19/50 (38%)
Adjusted rate	43.1%	33.4%	60.9%	60.0%
Terminal rate	12/30 (40%)	9/34 (26%)	14/25 (56%)	15/27 (56%)
First incidence (days)	611	440	464	484
Logistic regression test	P=0.030	P=0.445N	P=0.124	P=0.103

(continued)

TABLE 18
Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory Tract in Mice in the 2-Year Inhalation Study of Ozone (continued)

Dose (ppm)	0	0.12	0.5	1.0
Female				
Larynx	50	50	49	50
Epiglottis, Hyperplasia	0	0	0	7** (1.0)
Nose	50	50	48	50
Lateral Wall, Hyaline Degeneration	5 (1.0)	18* (1.0)	48** (2.6)	50** (3.5)
Lateral Wall, Fibrosis	0	3 (1.8)	46** (1.8)	50** (2.7)
Lateral Wall, Hyperplasia	0	0	42** (1.9)	50** (2.5)
Lateral Wall, Inflammation, Suppurative	0	5 (1.0)	46** (1.7)	50** (2.1)
Lateral Wall, Metaplasia, Squamous	1 (1.0)	1 (1.0)	11** (1.5)	36** (2.2)
Olfactory Epithelium, Atrophy	4 (1.8)	1 (1.0)	14* (1.5)	41** (1.8)
Lung	50	50	49	50
Alveolar Epithelium, Metaplasia	0	0	43** (1.5)	49** (2.6)
Alveolus, Infiltration Cellular, Histiocyte	0	0	11** (1.0)	42** (1.8)
Alveolar Epithelium, Hyperplasia	2 (2.0)	1 (4.0)	1 (1.0)	2 (2.0)
Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	5/50 (10%)	5/49 (10%)	8/50 (16%)
Adjusted rate	12.5%	12.9%	13.4%	20.0%
Terminal rate	3/29 (10%)	4/37 (11%)	2/33 (6%)	8/40 (20%)
First incidence (days)	636	681	667	735 (T)
Logistic regression test	P=0.153	P=0.549	P=0.515	P=0.239
Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	5/49 (10%)	8/50 (16%)
Adjusted rate	6.9%	5.2%	14.1%	19.2%
Terminal rate	2/29 (7%)	1/37 (3%)	3/33 (9%)	7/40 (18%)
First incidence (days)	735 (T)	703	709	488
Logistic regression test	P=0.011	P=0.649N	P=0.259	P=0.053
Alveolar/bronchiolar Adenoma or Carcinoma[i]				
Overall rate	6/50 (12%)	7/50 (14%)	9/49 (18%)	16/50 (32%)
Adjusted rate	19.2%	17.7%	24.0%	38.8%
Terminal rate	5/29 (17%)	5/37 (14%)	5/33 (15%)	15/40 (38%)
First incidence (days)	636	681	667	488
Logistic regression test	P=0.005	P=0.571	P=0.326	P=0.022

* Significantly different (P≤0.05) than the control group by the logistic regression test
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals (1=minimal; 2=mild; 3=moderate; 4=marked).
[d] Number of animals with neoplasm per number of animals necropsied
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal sacrifice
[g] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression tests regard these lesions as nonfatal. A lower incidence in an exposure group is indicated by N.
[h] Historical incidence for 2-year inhalation studies with untreated control groups (mean ± standard deviation): 150/673 (22.3% ± 9.0); range, 10%-42%
[i] Historical incidence: 58/659 (8.8 ± 3.5); range, 0%-15%

LIFETIME STUDY

Survival

Estimates of survival probabilities for male and female mice exposed to ozone by inhalation for 130 weeks are presented in Table 19 and in Kaplan-Meier survival curves (Figure 9). Survival rates of exposed mice were similar to those of the controls.

Body Weights and Clinical Findings

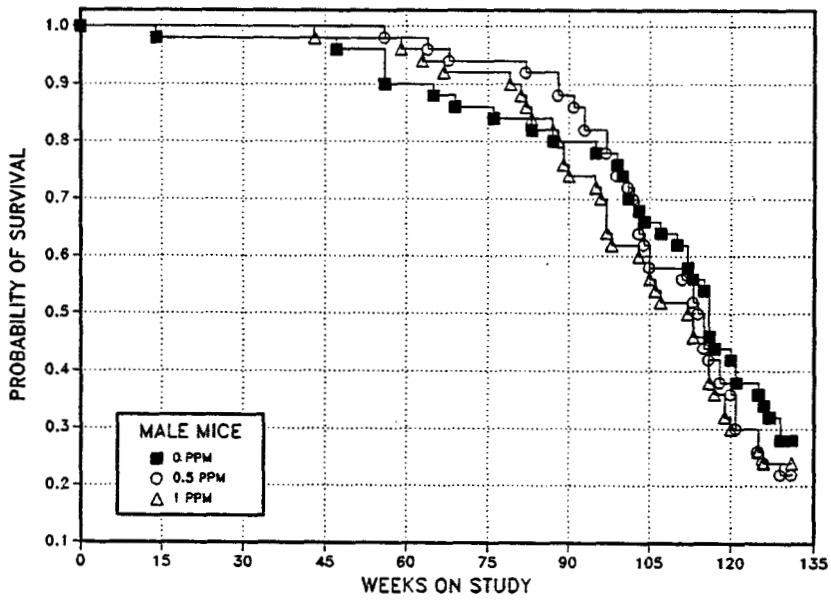
The mean body weights of 1.0 ppm mice, particularly those of 1.0 ppm females, were lower than those of the controls throughout most of the study (Tables 20 and 21 and Figure 10). Due to a laboratory error, feeders for control females were not replaced on the day prior to the week 113 weighing; this resulted in a marked weight loss for control females at week 113. However, the final mean body weights of all exposed groups were similar to those of the controls.

Hypoactivity was observed in male and female mice exposed to ozone. Mice, particularly those exposed to 1.0 ppm ozone, were less active during and immediately after exposure.

TABLE 19
Survival of Mice in the Lifetime Inhalation Study of Ozone

	0 ppm	0.5 ppm	1.0 ppm
Male			
Animals initially in study	50	50	50
Moribund	26	30	23
Natural deaths	10	9	15
Animals surviving to study termination	14	11	12
Percent probability of survival at end of study[a]	28	22	24
Mean survival (days)[b]	752	770	743
Survival analysis[c]	P=0.440	P=0.603	P=0.519
Female			
Animals initially in study	50	50	50
Moribund	34	25	33
Natural deaths	7	13	7
Animals surviving to study termination	9	12	10
Percent probability of survival at end of study	18	24	20
Mean survival (days)	775	804	769
Survival analysis	P=1.000N	P=0.377N	P=1.000N

[a] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A negative trend or a lower mortality in an exposure group is indicated by N.



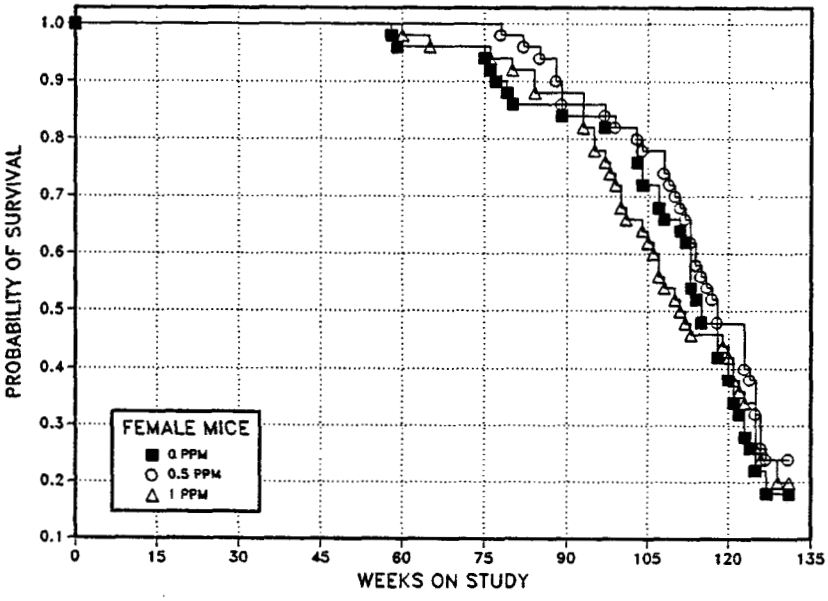


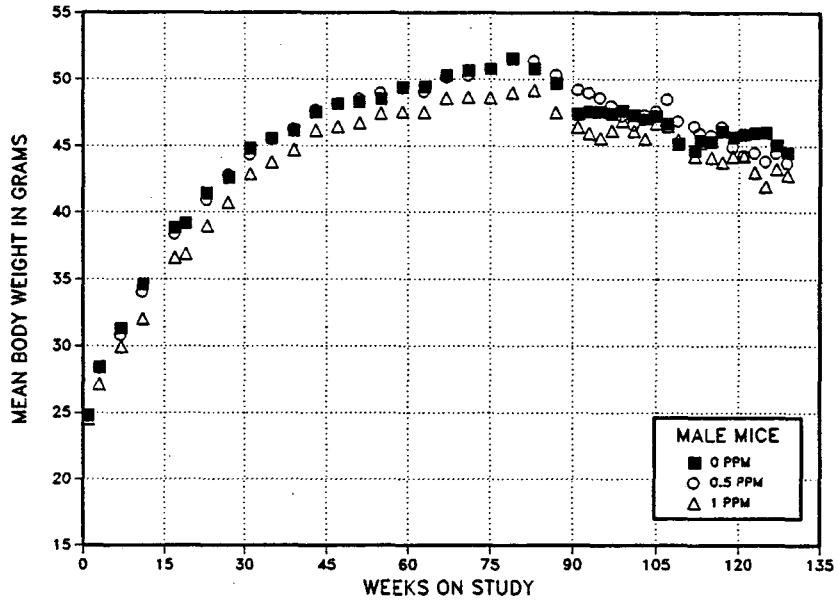
FIGURE 9
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Ozone by Inhalation for 130 Weeks

TABLE 20
Mean Body Weights and Survival of Male Mice in the Lifetime Inhalation Study of Ozone

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm Number of Survivors	0.5 ppm Av. Wt. (g)	0.5 ppm Wt. (% of controls)	0.5 ppm Number of Survivors	1.0 ppm Av. Wt. (g)	1.0 ppm Wt. (% of controls)	1.0 ppm Number of Survivors
1	24.8	50	24.7	100	50	24.5	99	50
3	28.4	50	28.3	100	50	27.1	95	50
7	31.3	50	30.8	98	50	29.9	96	50
11	34.6	50	34.1	99	50	32.0	93	50
17	38.8	49	38.4	99	50	36.6	94	50
19	39.2	49	39.1	100	50	36.9	94	50
23	41.4	49	40.9	99	50	39.0	94	50
27	42.6	49	42.8	101	50	40.7	96	50
31	44.8	49	44.3	99	50	42.9	96	50
35	45.5	49	45.5	100	50	43.8	96	50
39	46.2	49	46.3	100	50	44.7	97	50
43	47.5	49	47.7	100	50	46.2	97	49
47	48.2	48	48.2	100	50	46.5	97	49
51	48.3	48	48.6	101	50	46.7	97	49
55	48.5	48	49.0	101	50	47.5	98	49
59	49.4	45	49.4	100	49	47.6	96	48
63	49.5	45	49.1	99	49	47.5	96	47
67	50.3	44	50.2	100	48	48.6	97	46
71	50.7	43	50.3	99	47	48.7	96	46
75	50.8	43	50.8	100	47	48.6	96	46
79	51.6	42	51.6	100	47	49.0	95	45
83	50.8	41	51.4	101	46	49.2	97	43
87	49.7	40	50.3	101	46	47.6	96	42
91	47.4	40	49.2	104	43	46.4	98	37
93	47.6	40	49.0	103	42	46.0	97	37
95	47.6	40	48.6	102	41	45.6	96	37
97	47.4	39	48.0	101	39	46.1	97	32
99	47.6	39	47.3	99	39	46.9	99	31
101	47.3	36	46.6	99	37	46.1	98	31
103	47.0	35	47.3	101	33	45.5	97	30
105	47.2	33	47.6	101	31	46.7	99	28
107	46.7	33	48.5	104	29	46.5	100	27
109	45.2	32	46.9	104	29	45.5	101	26
112	44.7	31	46.5	104	28	44.3	99	26
113	45.4	29	46.0	101	27	45.2	100	24
115	45.4	28	45.8	101	23	44.2	97	23
117	46.1	22	46.4	101	21	43.8	95	19
119	45.7	22	44.9	98	19	44.2	97	16
121	45.9	20	44.3	97	17	44.3	97	15
123	46.0	19	44.5	97	15	43.1	94	15
125	46.0	18	43.9	95	14	42.0	91	14
127	45.1	16	44.5	99	12	43.3	96	12
129	44.5	15	43.7	98	11	42.8	96	12
Mean for weeks								
1-13	29.8		29.5	99		28.4	95	
14-52	44.3		44.2	100		42.4	96	
53-129	47.5		47.6	100		46.0	97	

TABLE 21
Mean Body Weights and Survival of Female Mice in the Lifetime Inhalation Study of Ozone

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm Number of Survivors	0.5 ppm Av. Wt. (g)	0.5 ppm Wt. (% of controls)	0.5 ppm Number of Survivors	1.0 ppm Av. Wt. (g)	1.0 ppm Wt. (% of controls)	1.0 ppm Number of Survivors
1	20.1	50	20.0	100	50	19.9	99	50
3	23.6	50	23.6	100	50	23.1	98	50
7	25.2	50	25.7	102	50	25.0	99	50
11	27.2	50	28.2	104	50	26.3	97	50
17	30.8	50	31.8	103	50	30.3	98	50
19	31.4	50	33.4	106	50	30.7	98	50
23	33.9	50	35.5	105	50	32.7	97	50
27	35.7	50	37.7	106	50	34.0	95	50
31	38.4	50	38.9	101	50	34.9	91	50
35	39.6	50	40.3	102	50	35.5	90	50
39	40.9	50	41.5	102	50	37.3	91	50
43	43.1	50	42.7	99	50	37.8	88	50
47	44.3	50	44.1	100	50	39.0	88	50
51	45.1	50	45.3	100	50	39.5	88	50
55	44.8	50	45.2	101	50	39.2	88	50
59	47.5	48	47.4	100	50	40.9	86	50
63	48.6	48	47.7	98	50	42.1	87	49
67	50.0	48	48.9	98	50	43.1	86	48
71	50.5	48	49.7	98	50	44.0	87	48
75	51.4	47	50.3	101	50	45.2	88	48
79	51.6	44	51.4	100	49	45.5	88	47
83	52.6	43	52.0	99	48	47.0	89	46
87	52.1	43	50.5	97	47	45.7	88	44
91	50.7	42	49.7	98	43	44.2	87	44
93	50.2	42	49.2	98	43	43.6	87	42
95	49.8	42	48.8	98	43	42.7	86	40
97	49.9	41	48.6	97	42	43.3	87	38
99	49.1	41	48.6	99	41	43.2	88	37
101	48.5	41	47.9	99	41	44.3	91	33
103	47.6	40	47.1	99	41	43.2	91	33
105	47.8	36	47.3	99	39	44.4	93	32
107	47.2	36	48.3	102	39	44.5	94	30
109	46.6	33	47.3	102	37	44.6	96	27
112	45.9	32	47.8	104	34	43.8	95	25
113	42.6	30	47.3	111	31	44.8	105	23
115	44.1	25	45.5	103	29	43.9	100	23
117	44.2	24	46.2	105	26	43.6	99	23
119	44.3	21	45.3	102	24	43.1	97	23
121	43.9	18	44.7	102	24	42.4	97	21
123	44.3	14	43.9	99	21	41.8	94	17
125	43.6	12	44.3	102	17	42.4	97	13
127	43.4	9	43.5	100	12	41.3	95	12
129	42.3	9	42.2	100	12	40.2	95	12
Mean for weeks								
1-13	24.0		24.4	102		23.6	98	
14-52	38.3		39.1	102		35.2	92	
53-129	47.4		47.5	100		43.4	92	



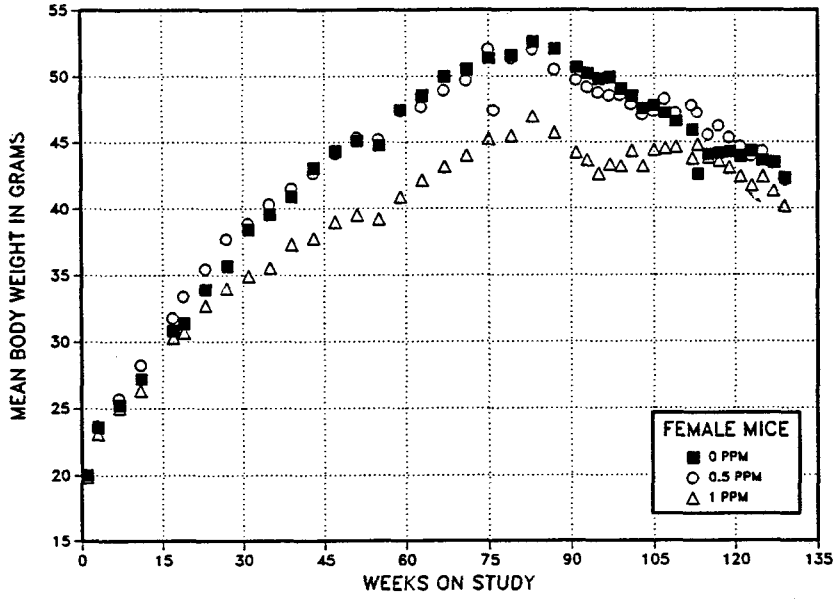


FIGURE 10
Growth Curves for Male and Female Mice Exposed to Ozone by Inhalation for 130 Weeks

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions in the lung, nose, and larynx. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal respiratory system tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix H for male mice and Appendix I for female mice.

Lung: Increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) occurred in exposed groups of males and females (Table 22). Although the increases were not statistically significant, the incidences increased with increasing ozone exposure. The incidence of carcinoma in exposed males was significantly greater than that in the controls. The incidence of adenoma in 1.0 ppm females was significantly greater than that in the controls. Multiple carcinomas occurred in male mice (0 ppm, 2/49; 0.5 ppm, 5/49; 1.0 ppm, 4/50; Table H1), and six high-dose males had both adenoma and carcinoma. When incidences of alveolar/bronchiolar adenoma or carcinoma (combined) from the 2-year and lifetime studies are considered together, the significance of the combined alveolar/bronchiolar adenoma or carcinoma incidences increases (Table 23). The use of historical controls from the NTP 2-year historical database is not applicable for these lifetime studies.

Metaplasia of the cuboidal (ciliated and nonciliated) epithelium was observed in the alveolar ducts with a minimal histiocytic infiltrate (Table 22). There were decreased incidences of hyperplasia in males and the incidences in females were similar to that of the controls.

Nose: Increased incidences of fibrosis, hyperplasia, and degeneration occurred in groups of males and females exposed to 0.5 or 1.0 ppm ozone, and an increased incidence of squamous metaplasia occurred in 1.0 ppm males and females (Table 22). The hyaline degeneration was characterized by brightly eosinophilic globules of varying size in the cytoplasm of epithelial cells lining the nasal passage and was similar to that observed in the 2-year study. Fibrosis was characterized by increased numbers of fibroblasts and collagen in the mucosa and was predominantly found in 1.0 ppm mice. The hyperplasia of the transitional epithelium along the lateral wall was similar to that seen in the 2-year study, as was the squamous metaplasia observed in 1.0 ppm mice.

Larynx: Squamous metaplasia of the epithelium at the base of the epiglottis occurred in mice and the incidences were greatest in 1.0 ppm males and females (Table 22). The increased incidence of this lesion was considered to be related to ozone exposure. The lesion was characterized by flattened cells which replaced normal cuboidal epithelium.

TABLE 22
Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory Tract in Mice in the Lifetime Inhalation Study of Ozone

Dose (ppm)	0	0.5	1.0
Male			
Larynx[a]	49	49	50
Hyperplasia[b]	4 (1.0)[c]	7 (1.3)	15**(1.1)
Epiglottis, Metaplasia, Squamous	2 (1.0)	1 (1.0)	10** (1.1)
Nose	49	48	49
Lateral Wall, Hyaline Degeneration	2 (1.5)	48** (1.1)	49** (2.5)
Lateral Wall, Fibrosis	0	8** (1.0)	43** (1.3)
Lateral Wall, Hyperplasia	2 (1.0)	33** (1.1)	45** (1.8)
Lateral Wall, Inflammation, Suppurative	1 (1.0)	38** (1.0)	46** (1.3)
Lateral Wall, Metaplasia, Squamous	1 (1.0)	2 (1.5)	20**(1.2)
Olfactory, Epithelium, Atrophy	4 (1.8)	4 (2.3)	18**(1.7)
Lung	49	49	50
Alveolar Epithelium, Metaplasia	0	48** (1.5)	47** (2.2)
Alveolus, Infiltration Cellular, Histiocyte	3 (3.0)	40** (1.8)	41** (1.7)
Alveolar Epithelium, Hyperplasia	10 (2.8)	8 (3.3)	1** (4.0)
Alveolar/bronchiolar Adenoma			
Overall rate[d]	8/49 (16%)	8/49 (16%)	9/50 (18%)
Adjusted rate[e]	33.9%	32.8%	50.6%
Terminal rate[f]	3/14 (21%)	2/11 (18%)	5/12 (42%)
First incidence (days)	391	678	620
Logistic regression test[g]	P=0.427	P=0.606N	P=0.473
Alveolar/bronchiolar Carcinoma			
Overall rate	8/49 (16%)	15/49 (31%)	18/50 (36%)
Adjusted rate	42.3%	65.3%	70.9%
Terminal rate	4/14 (29%)	5/11 (45%)	6/12 (50%)
First incidence (days)	805	693	609
Logistic regression test	P=0.005	P=0.050	P=0.007
Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	16/49 (33%)	22/49 (45%)	21/50 (42%)
Adjusted rate	66.0%	76.3%	77.0%
Terminal rate	7/14 (50%)	6/11 (55%)	7/12 (58%)
First incidence (days)	391	678	609
Logistic regression test	P=0.127	P=0.140	P=0.149

(continued)

TABLE 22
Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory Tract in Mice in the Lifetime Inhalation Study of Ozone (continued)

Dose (ppm)	0	0.5	1.0
Female			
Larynx	50	49	50
Hyperplasia	13 (1.2)	11 (1.3)	24* (1.3)
Epiglottis, Metaplasia, Squamous	2 (1.5)	2 (1.0)	19** (1.1)
Nose	50	49	50
Lateral Wall, Hyaline Degeneration	0	49** (2.0)	50** (2.4)
Lateral Wall, Fibrosis	1 (1.0)	23** (1.1)	48** (1.2)
Lateral Wall, Hyperplasia	1 (1.0)	42** (1.9)	47** (2.0)
Lateral Wall, Inflammation, Suppurative	3 (1.0)	44** (1.0)	50** (1.3)
Lateral Wall, Metaplasia, Squamous	2 (1.0)	3 (1.0)	28** (1.4)
Olfactory Epithelium, Atrophy	9 (1.4)	23* (1.9)	40** (2.2)
Lung	50	49	50
Alveolar Epithelium, Metaplasia	0	43** (1.0)	50** (2.1)
Alveolus, Infiltration Cellular, Histiocyte	5 (2.2)	39** (1.3)	45** (1.8)
Alveolar Epithelium, Hyperplasia	3 (1.7)	1 (2.0)	3 (3.0)
Alveolar/bronchiolar Adenoma			
Overall rate	3/50 (6%)	3/49 (6%)	11/50 (22%)
Adjusted rate	15.7%	8.9%	56.1%
Terminal rate	1/9 (11%)	0/12 (0%)	4/10 (40%)
First incidence (days)	721	616	455
Logistic regression test	P=0.009	P=0.633	P=0.020
Alveolar/bronchiolar Carcinoma			
Overall rate	3/50 (6%)	5/49 (10%)	2/50 (4%)
Adjusted rate	12.2%	26.4%	13.9%
Terminal rate	0/9 (0%)	2/12 (17%)	1/10 (10%)
First incidence (days)	521	721	833
Logistic regression test	P=0.423N	P=0.328	P=0.496N
Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	6/50 (12%)	8/49 (16%)	12/50 (24%)
Adjusted rate	26.0%	33.1%	58.0%
Terminal rate	1/9 (11%)	2/12 (17%)	4/10 (40%)
First incidence (days)	521	616	455
Logistic regression test	P=0.072	P=0.341	P=0.096

* Significantly different (P≤0.05) from the control group by the logistic regression test
** P≤0.01
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals (1=minimal; 2=mild; 3=moderate; 4=marked).
[d] Number of animals with neoplasm per number of animals necropsied
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence at terminal sacrifice
[g] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards these lesions as nonfatal. A negative trend or a lower incidence in an exposure group is indicated by N.

TABLE 23
Incidences of Alveolar/bronchiolar Neoplasms in Mice in the 2-Year and Lifetime Inhalation Studies of Ozone (Combined Analysis)

Dose (ppm)	0	0.5	1.0
Male			
Alveolar/bronchiolar Adenoma			
Overall rate[a]	14/99 (14%)	20/99 (20%)	20/100 (20%)
Adjusted rate[b]	38.5%	44.2%	59.1%
2-Year sacrifice[c]	5/29 (17%)	9/25 (36%)	8/27 (30%)
Terminal rate[d]	3/14 (21%)	2/11 (18%)	5/12 (42%)
First incidence (days)	391	464	484
Logistic regression test[e]	P=0.132	P=0.164	P=0.143
Alveolar/bronchiolar Carcinoma			
Overall rate	16/99 (16%)	23/99 (23%)	28/100 (28%)
Adjusted rate	49.8%	69.6%	75.3%
2-Year sacrifice	7/29 (24%)	7/25 (28%)	9/27 (33%)
Terminal rate	4/14 (29%)	5/11 (45%)	6/12 (50%)
First incidence (days)	653	693	609
Logistic regression test	P=0.006	P=0.085	P=0.009
Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	30/99 (30%)	40/99 (40%)	40/100 (40%)
Adjusted rate	72.7%	82.2%	83.2%
2-Year sacrifice	12/29 (41%)	14/25 (56%)	15/27 (56%)
Terminal rate	7/14 (50%)	6/11 (55%)	7/12 (58%)
First incidence (days)	391	464	484
Logistic regression test	P=0.037	P=0.058	P=0.045

(continued)

TABLE 23
Incidences of Alveolar/bronchiolar Neoplasms in Mice in the 2-Year and Lifetime Inhalation Studies of Ozone (Combined Analysis) (continued)

Dose (ppm)	0	0.5	1.0
Female			
Alveolar/bronchiolar Adenoma			
Overall rate	7/100 (7%)	8/98 (8%)	19/100 (19%)
Adjusted rate	19.1%	13.8%	60.7%
2-Year sacrifice	3/29 (10%)	2/33 (6%)	8/40 (20%)
Terminal rate	1/9 (11%)	0/12 (0%)	4/10 (40%)
First incidence (days)	636	616	455
Logistic regression test	P=0.005	P=0.475	P=0.010
Alveolar/bronchiolar Carcinoma			
Overall rate	5/100 (5%)	10/98 (10%)	10/100 (10%)
Adjusted rate	13.5%	31.3%	24.6%
2-Year sacrifice	2/29 (7%)	3/33 (9%)	7/40 (18%)
Terminal rate	0/9 (0%)	2/12 (17%)	1/10 (10%)
First incidence (days)	521	709	488
Logistic regression test	P=0.126	P=0.140	P=0.139
Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	12/100 (12%)	17/98 (17%)	28/100 (28%)
Adjusted rate	30.1%	40.1%	67.2%
2-Year sacrifice	5/29 (17%)	5/33 (15%)	15/40 (38%)
Terminal rate	1/9 (11%)	2/12 (17%)	4/10 (40%)
First incidence (days)	521	616	455
Logistic regression test	P=0.003	P=0.197	P=0.004

[a] Number of animals with neoplasm per number of animals necropsied
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence in animals sacrificed at the end of the 2-year study
[d] Observed incidence at the end of the lifetime study
[e] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards these lesions as nonfatal.

GENETIC TOXICOLOGY

Concurrent dosimetry was conducted with each trial because, as shown in Table J1, identical voltage and oxygen flow parameters did not ensure identical ozone concentrations. Generation of ozone from oxygen was not 100% efficient and some residual oxygen was presumably present in the exposure jar atmospheres, but the amount could not be quantified. Therefore, statistical analyses presented in Table J1 are from comparisons with air controls only, although the data for the oxygen controls are included. Comparison of the individual dose points to the oxygen control values reduced the significance of some of the responses, but did not change a mutagenic response to a nonmutagenic response in any of the experiments (see Dillon *et al.*, 1992).

No induction of mutations was observed in experiments conducted with an oxygen flow rate of 5 L/minute with strains TA98, TA100, TA104, or TA1535, (data not shown; see Dillon *et al.*, 1992). Positive responses were obtained with strain TA102, however, in all four experiments conducted, two with oxygen flow rates of 5 L/minute and two with flow rates of 7 L/minute; the data presented in Table J1 are from the second set of experiments (Dillon *et al.*, 1992). The same voltage settings were used in all experiments. In most experiments, similar results were obtained with and without S9. The positive responses occurred at the lower voltages (100, 125, and 132 volts); higher voltages, that produced higher concentrations of ozone, resulted in increasing toxicity and decreases in the numbers of mutant colonies.

PLATE 1
Clusters of goblet cells (arrows) within the respiratory epithelium of the nasoturbinates in a male F344/N rat exposed to 1.0 ppm ozone for 2 years. H&E; 280×

PLATE 2
Centriacinar region of the lung from a female F344/N rat exposed to 1.0 ppm ozone for 2 years. There is a cluster of macrophages (arrow) at the bifurcation of the terminal bronchiole (TB) and also thickening of the epithelium in the alveolar duct (arrowheads). H&E; 120×

PLATE 3
Cuboidal cells (arrows) occurring between alveoli in the alveolar duct of a female F344/N rat exposed to 1.0 ppm ozone for 2 years. H&E; 210×

PLATE 4
Alveolar/bronchiolar adenoma from a control male $B6C3F_1$ mouse (arrows). The size and morphology of pulmonary neoplasms was similar in control animals and in animals exposed to ozone. H&E; 28×

DISCUSSION AND CONCLUSIONS

Ozone is the major oxidizing component in polluted air found in many urban environments. Exposure to ozone, a highly reactive toxic molecule, causes a wide variety of effects in laboratory animals (Boorman *et al.*, 1980; Eustis *et al.*, 1981; Hatch *et al.*, 1986; USEPA, 1986; Graham and Koren, 1990; Rajini *et al.*, 1993). Ozone levels which have been found in the environment cause lung inflammation, acute changes in lung function, and alterations in pulmonary structure. Changes in pulmonary function and increased numbers of inflammatory cells in pulmonary lavage fluid are also seen in humans (Koren *et al.*, 1991). The state of California and the Health Effects Institute (a nonprofit institute supported by the U.S. Environmental Protection Agency and the automobile industry) nominated ozone to the National Toxicology Program for evaluation in long-term rodent studies because of the lack of adequate information on chronic toxicity and potential carcinogenicity.

Concentrations of ozone ranging from those found in urban environments to maximum tolerated doses were used to study the toxic effects of long-term ozone exposure and to examine the effects of ozone using concentrations similar to levels at which humans may be exposed. Because rodent pulmonary neoplasms often occurred after 2 years in the diesel exhaust studies, lifetime as well as 2-year studies were included. Finally, to determine whether ozone could promote pulmonary neoplasms, a study was included in which male rats were administered 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a known pulmonary carcinogen, in addition to ozone.

Because many short-term studies have analyzed the biological effects of ozone and because differences in sensitivity to ozone among rodent strains may exist, a 4-week study was conducted to determine if F344/N rats and B6C3F$_1$ mice could tolerate the highest ozone concentrations selected for these studies. A spectrum of lesions similar to those observed in other strains of rats and mice occurred in the 4-week studies; results from the 4-week studies also indicated that 1.0 ppm ozone, the highest concentration chosen, was not likely to affect long-term survival. Because marked pulmonary edema has been observed in rats and mice exposed to 2 ppm ozone for 4 hours (Hatch *et al.*, 1986), 1.0 ppm was considered to be the highest tolerable dose for F344/N rats and B6C3F$_1$ mice.

The mean body weights of male and female rats and male mice exposed to 1.0 ppm were generally 5% to 8% lower than those of the controls throughout the 2-year and lifetime studies; mean body weights of female mice were 10% lower than that of the controls for most of the study. The mean body weights of rats and mice exposed to 0.12 or 0.5 ppm ozone were similar to those of the controls throughout the 2-year and lifetime studies.

Exposure to ozone appeared to have little effect on survival rates of rats and mice. This would suggest the ozone toxicity was not having a marked effect even though the highest doses were close to lethal concentrations.

In the present studies, toxicity observed in the pulmonary airways was similar to that observed following short-term ozone exposures (Boorman *et al.*, 1980; Hatch *et al.*, 1989; Pinkerton *et al.*, 1992), but with some notable differences. As in previous studies, the lesions tended to predominate mostly in the centriacinar region of the lung, an area that is known to be especially sensitive to the toxic effects of ozone. Both rats and mice exposed to ozone for 2 years or longer had increased numbers of inflammatory cells in the centriacinar region and an extension of the ciliated and nonciliated (Clara) bronchial cells into the alveolar ducts. Interstitial centriacinar fibrosis of the septa was observed histopathologically in rats at the end of the 2-year study and, more prominently, at the end of the lifetime study; this lesion is not as prominent (histologically) with exposures of 3 months or less. Interstitial fibrosis was diagnosed in all rats exposed to 1.0 ppm ozone in the lifetime study and in 85% or more of the 1.0 ppm rats in the 2-year study. This fibrous change was not observed in mice. Fibrosis is generally not recognized histopathologically following short-term ozone exposures, but has been documented ultrastructurally using

morphological techniques in rats exposed to peak levels of 0.25 ppm ozone for 13 and 78 weeks (Chang *et al.*, 1992).

Adaptation is a term that has been used to refer to the decreases in inflammatory response and in cellular necrosis that occur with prolonged exposure to ozone (Schwartz *et al.*, 1976; Hotchkiss *et al.*, 1989). With continuous exposures of up to 30 months, basic centriacinar ozone-induced lesions persist, and ultrastructurally the lesions are more advanced than those that develop following shorter exposure periods (Pinkerton *et al.*, 1993). This suggests that while there is adaptation in the sense that the inflammatory response subsides, the degree of cell necrosis falls with time, and cell proliferation levels drop, there continues to be remodeling and fibrosis with continuing exposure. Thus, the effects of long-term exposures would be overestimated using short-term exposures in animal models. Similarly, assuming that animals and man can adapt to ambient ozone levels may underestimate the potential hazard of long-term ozone exposures. The current studies suggest that continuous exposure to ozone over long periods of time may be expected to have cumulative adverse effects.

For policymakers, ozone concentration-response decisions are especially problematic because levels of 0.1 to 0.5 ppm exist in the environment and toxic changes are seen in rodents at these levels. In addition, levels of 0.1 to 0.5 ppm are within an order of magnitude of the lethal dose for some species (2 to 3 ppm). Increased incidences of inflammation or extension of bronchial epithelial cells (metaplasia) into the centriacinar region were observed in mice exposed to 0.5 or 1.0 ppm ozone, but not in mice exposed to 0.12 ppm ozone. While the incidences of inflammation and metaplasia in mice exposed to 0.5 or 1.0 ppm were similar, the severities were greater in the 1.0 ppm groups. Incidences of mild metaplasia were observed in 0.12 ppm male and female rats. These results suggest that the dose-response curve for ozone is very steep. Further, because adaptation occurs during acute exposures and some remodeling and fibrosis occur during long-term exposures, the concentration/time relationships for ozone toxicity are very complex.

The ozone dose-response relationship is less clear when nasal passage lesions are evaluated. In the present studies, an increase in the incidence of hyperplasia of the noncuboidal epithelium (transitional epithelium) along the lateral wall of the nasal passage occurred in rats. In addition, there was an increase in the incidence of squamous metaplasia of the epithelium in the anterior portion of the nasal passage. Increased incidences of inflammation of the nasal passage were observed in mice exposed to 0.12 ppm, suggesting that even at 0.12 ppm, ozone has a toxic effect on the epithelium lining the nasal passage. No treatment-related neoplasms were observed in the nasal passages of rats or mice. This suggests that the hyperplasia occurring in the transitional epithelium of the nasal cavity after ozone exposure has little propensity to progress to neoplasia even after 30 months of exposure (Johnson *et al.*, 1990).

While the toxicity of ozone to the respiratory passages of animals and humans has been well described, the potential of this reactive compound to affect the carcinogenic process is less clear. Ozone is mutagenic in *Salmonella typhimurium* (Dillon *et al.*, 1992) and has been reported to be carcinogenic in mice (Hassett *et al.*, 1985; Last *et al.*, 1987) but not in other species studied. The present studies suggest that ozone is not carcinogenic in the F344/N rat. Pulmonary neoplasms are less common in female rats than in male rats. In the 2-year study, two alveolar/bronchiolar carcinomas were observed in female rats exposed to 0.5 ppm ozone while no alveolar/bronchiolar carcinomas were observed in the control, 0.12, or 1.0 ppm groups. One alveolar/bronchiolar adenoma and one alveolar/bronchiolar carcinoma were observed in 2-year control males, and two alveolar/bronchiolar adenomas were observed in lifetime control males. No male exposure group had more than four alveolar/bronchiolar adenomas or carcinomas (combined). While three alveolar/bronchiolar adenomas and one alveolar/bronchiolar carcinoma were observed in 1.0 ppm male rats from the 2-year study, no alveolar/bronchiolar adenomas or carcinomas were observed in 1.0 ppm males from the lifetime study. This lack of consistency argues against even a marginal effect of ozone on the incidence of pulmonary neoplasms in the F344/N rat. Another study in rats showed that ozone exposure alone had no effect on pulmonary neoplasms (Ichinose and Sagai, 1992).

Ozone did not enhance the carcinogenic effect of NNK (a tobacco-specific nitrosamine) in rats. Rats exposed to 0.1 mg NNK/kg body weight and 0.5 ppm

ozone had three alveolar/bronchiolar adenomas; two alveolar/bronchiolar adenomas were observed in rats exposed to 0.1 mg/kg NNK without ozone. Alveolar/bronchiolar adenomas were observed in 23 rats exposed to 1.0 mg/kg NNK and 0.5 ppm ozone; 20 rats exposed to 1.0 mg/kg NNK and 0 ppm ozone had alveolar/bronchiolar adenomas. The incidence of pulmonary carcinomas was also similar between NNK/ozone rats and rats exposed only to NNK. Eleven alveolar/bronchiolar carcinomas were observed in rats exposed to 1.0 mg/kg NNK and 0.5 ppm ozone, and eight alveolar/bronchiolar carcinomas were observed in rats exposed to 1.0 mg/kg NNK alone. It is not known whether different results would have been obtained with a different carcinogenic initiator. Ichinose and Sagai (1992) have suggested that *N*-bis(2-hydroxypropyl) nitrosamine (BHPN) pulmonary tumorigenesis can be enhanced by ozone exposure, but the enhancement only occurred when ozone was administered in combination with nitrogen dioxide.

Previous studies have suggested that ozone exposure can enhance the carcinogenic process in mice (Hassett *et al.*, 1985). In the present studies, mice were administered up to 1.0 ppm ozone for 2 years or 130 weeks. There was a tendency toward increased incidences of pulmonary neoplasms with increasing ozone concentrations, but some inconsistencies were observed. In the 2-year study, a more dramatic effect was observed in female mice, primarily due to increased incidences of alveolar/bronchiolar carcinomas; two carcinomas were observed in controls and eight were observed in 1.0 ppm females. In males, there was a slight increase in the number of adenomas and the total number of neoplasm-bearing animals. A significant positive trend in the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) was also observed in males in the 2-year study (0 ppm, 14/50; 0.12 ppm, 13/50; 0.5 ppm, 18/50; 1.0 ppm, 19/50). In the lifetime study, a statistically significant increased incidence of alveolar/bronchiolar carcinomas occurred in 1.0 ppm males (0 ppm, 8/49; 0.5 ppm, 15/49; 1.0 ppm 18/50). There was no increased incidence of alveolar/bronchiolar carcinomas in female mice in the lifetime study, but an increased incidence of alveolar/bronchiolar adenomas did occur in 1.0 ppm females (3/50, 3/49, 11/50). When the incidences of pulmonary neoplasms in the 2-year and lifetime studies were combined the results were more significant. There was also some suggestion for increased multiplicity of neoplasms in male mice. It appears that the concordance between studies and between sexes in a tissue where ozone would be expected to have an effect is consistent with ozone-induced pulmonary neoplasia in mice. In contrast, there was little or no evidence that increasing exposure was associated with an increased incidence of neoplasia in rats.

Because pulmonary neoplasms in mice form a spectrum of lesions and adenomas appear to progress into carcinomas with time, it is useful to examine the total number of neoplasm-bearing mice. Using the parameter of the total number of neoplasm-bearing mice, results of the present studies appear to have greater consistency; in both studies, the number of 1.0 ppm females with alveolar/bronchiolar adenoma or carcinoma (combined) was approximately twice the number of control females observed to have the neoplasm (2-year study: 0 ppm, 6/50, and 1.0 ppm, 16/50; lifetime study: 6/50 and 12/50). The males also showed an increase, though less striking, in the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) (2-year study: 14/50 and 19/50; lifetime study: 16/49 and 21/50). Thus, there appears to be a consistent increase in the incidence of pulmonary neoplasms in mice with increasing ozone exposure, and it is more pronounced in females than in males.

In two studies with A/J mice, a strain that is highly susceptible to lung neoplasms, ozone exposure appeared to increase the incidence of pulmonary neoplasms (Hassett *et al.*, 1985; Last *et al.*, 1987). However, Last *et al.* (1987) found no increase in the incidence of pulmonary neoplasms in the Swiss Webster mouse. In each of these mouse strains, ozone exposure resulted in a decrease in the incidence of urethane-induced pulmonary neoplasms.

These studies support the observation that ozone increases the incidence of pulmonary neoplasms in a species (mouse) that is quite susceptible to pulmonary neoplasms. In these 2-year and lifetime NTP rat studies 1.0 ppm ozone had no effect on survival or on the incidence of pulmonary neoplasms, and further studies in rats could be predicted to be negative. The 13-month study with Wistar rats appears to confirm the lack of effect of ozone on pulmonary neoplasm incidence in rats (Ichinose and Sagai, 1992). Further study will be necessary to determine which is the most appropriate animal model for humans.

The toxic pulmonary lesion, metaplasia, occurred in both rats and mice. The continued inflammatory process and the increasing fibrosis suggests that these chronic toxic lesions may be important.

CONCLUSIONS

Under the conditions of these 2-year and lifetime inhalation studies, there was *no evidence of carcinogenic activity** of ozone in male or female F344/N rats exposed to 0.12, 0.5, or 1.0 ppm. There was *equivocal evidence of carcinogenic activity* of ozone in male $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. There was *some evidence of carcinogenic activity* of ozone in female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma.

There was no evidence that exposure to 0.5 ppm ozone enhanced the incidence of NNK-induced pulmonary neoplasms in male rats.

Exposure of male and female rats to ozone for 2 years or 125 weeks was associated with goblet cell hyperplasia and squamous metaplasia in the nose, squamous metaplasia in the larynx, and metaplasia (extension of bronchial epithelium into the centriacinar alveolar ducts) and interstitial fibrosis in the lung. Exposure of male and female mice to ozone for 2 years or 130 weeks was associated with hyperplasia and squamous metaplasia in the nose and inflammation (histiocytic infiltration) and metaplasia (extension of bronchial epithelium into the centriacinar alveolar ducts) of the lung.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

REFERENCES

Adams, W.C., Schelegle, E.S., and Shaffrath, J.D. (1989). Oral and oronasal breathing during continuous exercise produce similar responses to ozone inhalation. *Arch. Environ. Health* **44**, 311-316.

Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 362-365. John Wiley and Sons, New York.

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity, and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229-306.

Boehme, D.S., Hotchkiss, J.A., and Henderson, R.F. (1992). Glutathione and GSH-dependent enzymes in bronchoalveolar lavage fluid cells in response to ozone. *Exp. Mol. Pathol.* **56**, 37-48.

Boorman, G.A., Schwartz, L.W., and Dungworth, D.L. (1980). Pulmonary effects of prolonged ozone insult in rats. *Lab. Invest.* **43**, 108-115.

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

Burleson, G.R., Keyes, L.L., and Stutzman, J.D. (1989). Immunosuppression of pulmonary natural killer activity by exposure to ozone. *Immunopharmacol. Immunotoxicol.* **11**, 715-735.

Castleman, W.L., Dungworth, D.L., Schwartz, L.W., and Tyler, W.S. (1980). Acute respiratory bronchitis: An ultrastructural and autoradiographic study of epithelial cell injury and renewal in Rhesus monkeys exposed to ozone. *Am. J. Pathol.* **98**, 811-840.

Chang, L.Y., Huang, Y., Stockstill, B.L., Graham, J.A., Grose, E.C., Menache, M.G., Miller, F.J., Costa, D.L., and Crapo, J.D. (1992). Epithelial injury and interstitial fibrosis in the proximal alveolar regions of rats chronically exposed to a simulated pattern of urban ambient ozone. *Toxicol. Appl. Pharmacol.* **115**, 241-252.

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

Code of Federal Regulations (CFR), **40**, Part 50.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crapo, J.D., Barry, B.E., Chang, L.Y., and Mercer, R.R. (1984). Alterations in lung structure caused by inhalation of oxidants. *J. Toxicol. Environ. Health* **13**, 301-321.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology: Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific Publishing Co., Inc., Princeton, NJ.

Dillon, D., Combes, R., McConville, M., and Zeiger, E. (1992). Ozone is mutagenic in *Salmonella*. *Environ. Mol. Mutagen.* **19**, 331-337.

Dinse, G.E., and Haseman, J.K. (1986). Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. *Fundam. Appl. Toxicol.* **6**, 44-52.

Dinse, G.E., and Lagakos, S.W. (1983). Regression analysis of tumour prevalence data. *Appl. Statist.* **32**, 236-248.

Dungworth, D.L., Castleman, W.L., Chow, C.K., Mellick, P.W., Mustafa, M.G., Tarkington, B., and Tyler, W.S. (1975). Effect of ambient levels of ozone on monkeys. *Fed. Proc.* **34**, 1670-1674.

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

Elsayed, N.M., Kass, R., Mustafa, M.G., Hacker, A.D., Ospital, J.J., Chow, C.K., and Cross, C.E. (1988). Effect of dietary vitamin E level on the biochemical response of rat lung to ozone inhalation. *Drug-Nutr. Interact.* **5**, 373-386.

Erdman, H.E., and Hernandez, T. (1982). Adult toxicity and dominant lethals induced by ozone at specific stages in spermatogenesis in *Drosophila virilis*. *Environ. Mutagen.* **4**, 657-666.

Eustis, S.L., Schwartz, L.W., Kosch, P.C., and Dungworth, D.L. (1981). Chronic bronchitis in nonhuman primates after prolonged oxygen exposure. *Am. J. Path.* **105**, 121-137.

Fujinaka, L.G., Hyde, D.M., Plopper, C.G., Tyler, W.S., Dungworth, D.L., and Lollthi, L.O. (1985). Respiratory bronchitis following long term ozone exposure on Bonnet monkeys: A morphometric study. *Exp. Lung Res.* **8**, 167-190.

Gardner, D.E. (1982). Use of experimental airborne infections for monitoring altered host defenses. *Environ. Health Perspect.* **43**, 99-107.

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

Gerrity, T.R., Weaver, R.A., Berntsen, J., House, D.E., and O'Neil, J.J. (1988). Extrathoracic and intrathoracic removal of O_3 in tidal-breathing humans. *J. Appl. Physiol.* **65**, 393-400.

Gilmour, M.I., Park, P., Doerfler, D., and Selgrade, M.K. (1993). Factors that influence the suppression of pulmonary antibacterial defenses in mice exposed to ozone. *Exp. Lung Res.* **19**, 299-314.

Gong, H., Jr. (1992). Health effects of air pollution. *Clin. Chest Med.* **13**, 201-214.

Gooch, P.C., Creasia, D.A., and Brewen, J.G. (1976). The cytogenetic effects of ozone: Inhalation and *in vitro* exposures. *Environ. Res.* **12**, 188-195.

Graham, D.E., and Koren, H.S. (1990). Biomarkers of inflammation in ozone-exposed humans: Comparison of the nasal and bronchoalveolar lavage. *Am. Rev. Respir. Dis.* **142**, 152-156.

Grotberg, J.B. (1990). Gas absorption in pulmonary airways at low Peclet number. *J. Biomech. Eng.* **112**, 177-182.

Guerrero, R.R., Rounds, D.E., Olson, R.S., and Hackney, J.D. (1979). Mutagenic effects of ozone on human cells exposed *in vivo* and *in vitro* based on sister chromatid exchange analysis. *Environ. Res.* **18**, 336-346.

Hamelin, C., and Chung, Y.S. (1974). Optimal conditions for mutagenesis by ozone in *Escherichia coli* K12. *Mutat. Res.* **24**, 271-279.

Harkema, J.R., Plopper, C.G., Hyde, D.M., Wilson, D.W., St. George, J.A., and Wong, V.J. (1987). Nonolfactory surface epithelium of the nasal cavity of the Bonnet monkey: A morphologic and morphometric study of the transitional and respiratory epithelium. *Am. J. Anat.* **180**, 266-279.

Haseman, J.K. (1984). Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* **58**, 385-392.

Haseman, J.K., Huff, J., and Boorman, G.A. (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* **12**, 126-135.

Haseman, J.K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A., and McConnell, E.E. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N × C3H/HeN)F_1 (B6C3F_1) mice. *JNCI* **75**, 975-984.

Hassett, C., Mustafa, M.G., Coulson, W.F., and Elashoff, R.M. (1985). Murine lung carcinogenesis following exposure to ambient ozone concentrations. *JNCI* **75**, 771-777.

Hatch, G.E., Slade, R., Stead, A.G., and Graham, J.A. (1986). Species comparison of acute inhalation toxicity of ozone and phosgene. *J. Toxicol. Environ. Health* **19**, 43-53.

Hatch, G.E., Koren, H., and Aissa, M. (1989). Biological factors in modeling: Respiratory tract. A method for comparison of animal and human alveolar dose and toxic effect of inhales ozone. *Health Phys.* **57** (Suppl. 1), 37-40.

Hawley's Condensed Chemical Dictionary (1987). 11th ed. (N.I. Sax and R.J. Lewis, Sr., Eds.) pp. 866-867. Van Nostrand Reinhold Co., New York.

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

Hotchkiss, J.A., Harkema, J.R., Kirkpatrick, D.T., and Henderson, R.F. (1989). Response of rat alveolar macrophages to ozone: Quantitative assessment of population size, morphology, and proliferation following acute exposure. *Exp. Lung Res.* **15**, 1-16.

Hsueh, J.L., and Xiang, W. (1984). Environmental mutagenesis research at Fudan University. *Environ. Sci. Res.* **31**, 755-769.

Hu, S.C., Ben-Jebria, A., and Ultman, J.S. (1992). Longitudinal distribution of ozone absorption in the lung: Quiet respiration in healthy subjects. *J. Appl. Physiol.* **73**, 1655-1661.

Hynes, B., Silverman, F., Cole, P., and Corey, P. (1988). Effects of ozone exposure: A comparison between oral and nasal breathing. *Arch. Environ. Health* **43**, 357-360.

Ichinose, T., and Sagai, M. (1992). Combined exposure to NO_2, O_3, and H_2SO_4-aerosol and lung tumor formation in rats. *Toxicology* **74**, 173-184.

Johnson, N.F., Hotchkiss, J.A., Harkema, J.R., and Henderson, R.F. (1990). Proliferative responses of rat nasal epithelia to ozone. *Toxicol. Appl. Pharmacol.* **103**, 143-155.

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

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Koren, H.S., Devlin, R.B., Becker, S., Perez, R., and McDonnell, W.F. (1991). Time-dependent changes of markers associated with inflammation in the lungs of humans exposed to ambient levels of ozone. *Toxicol. Pathol.* **19**, 406-411.

Lai, C.C., Finlayson-Pitts, B.J., and Willis, W.V. (1990). Formation of secondary ozonides from the reaction of an unsaturated phosphatidylcholine with ozone. *Chem. Res. Toxicol.* **3**, 517-523.

Last, J.A., Warren, D.L., Pecquet-Goad, E., and Witschi, H. (1987). Modification by ozone of lung tumour development in mice. *JNCI* **78**, 149-154.

Li, A.F.-Y., and Richters, A. (1991). Ambient level ozone effects on subpopulations of thymocytes and spleen T lymphocytes. *Arch. Environ. Health* **46**, 57-63.

Lippmann, M. (1989). Health effects of ozone: A critical review. *J. Air Pollut. Cont. Assoc.* **39**, 672.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

McKenzie, W.H. (1982). Controlled human exposure studies: Cytogenetic effects of ozone inhalation. In *Indications of Gentoxic Exposure* (B.A. Bridges, B.E. Butterworth, and I.B. Weinstein, Eds.), pp. 319-324. Cold Spring Harbor Press, Cold Spring Harbor, NY.

McKenzie, W.H., Knelson, J.H., Rummo, N.J., and House, D.E. (1977). Cytogenetic effects of inhaled ozone in man. *Mutat. Res.* **48**, 95-102.

McKnight, B., and Crowley, J. (1984). Tests for differences in tumor incidence based on animal carcinogenesis experiments. *J. Am. Stat. Assoc.* **79**, 639-648.

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

Merz, T., Bender, M.A., Kerr, H.D., and Kulle, T.J. (1975). Observations of aberrations in chromosomes of lymphocytes from human subjects exposed to ozone at a concentration of 0.5 ppm for 6 and 10 hours. *Mutat. Res.* **31**, 299-302.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Miller, F.J., Overton, J.H., Jr., Jaskot, R.H., and Menzel, D.B. (1985). A model of the regional uptake of gaseous pollutants in the lung. I. The sensitivity of the uptake of ozone in the human lung to lower respiratory tract secretions and exercise. *Toxicol. Appl. Pharmacol.* **79**, 11-27.

Miller, F.J., Overton, J.H., Kimbell, J.S., and Russell, M.L. (1993). Regional respiratory tract absorption of inhaled reactive gases. In *Toxicology of the Lung* (D.E. Gardner, J.D. Crapo, and R.O. McClellan, Eds.), 2nd ed., pp. 485-525. Raven Press, New York.

Mittler, S., Hedrick, D., King, M., and Gaynor, A. (1956). Toxicity of ozone. I. Acute toxicity *Ind. Med. Surg.* **25**, 301-306.

Moore, P.F., and Schwartz, L.W. (1981). Morphological effects of prolonged exposure to ozone and sulfuric acid aerosol on the rat lung. *Exp. Mol. Pathol.* **35**, 108-123.

Mustafa, M.G. (1990). Biochemical basis of ozone toxicity. *Free Radic. Biol. Med.* **9**, 245-265.

Overton, J.H., and Graham, R.C. (1989). Predictions of ozone absorption in human lungs from newborn to adult. *Health Phys.* **57** (suppl. 1), 29-36.

Patty's Industrial Hygiene and Toxicology. (1985). 2nd ed. (L.J. Cralley and L.V. Cralley, Eds.). John Wiley and Sons, New York.

Pinkerton, K.E., Mercer, R.R., Plopper, C.G., and Crapo, J.D. (1992). Distribution of injury and microdosimetry of ozone in the ventilatory unit of the rat. *J. Appl. Physiol.* **73**, 817-824.

Pinkerton, K.E., Dodge, D.E., Cederdahl-Demmler, J., Wong, V.J., Peake, J., Haselton, C.J., Mellick, P.W., Singh, G., and Plopper, C.G. (1993). Differentiated bronchiolar epithelium in alveolar ducts of rats exposed to ozone for 20 months. *Am. J. Pathol.* **142**, 947-956.

Pryor, W.A. (1992). How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts? *Free Radic. Biol. Med.* **12**, 83-88.

Pryor, W.A., Das, B., and Church, D.F. (1991). The ozonation of unsaturated fatty acids: Aldehydes and hydrogen peroxide as products and possible mediators of ozone toxicity. *Chem. Res. Toxicol.* **4**, 341-348.

Rajini, P., Gelzleichter, T.R., Last, J.A., and Witschi, H. (1993). Alveolar and airway cell kinetics in the lungs of rats exposed to nitrogen dioxide, ozone, and a combination of the two gases. *Toxicol. Appl. Pharmacol.* **121**, 186-192.

Rithidech, K., Hotchkiss, J.A., Griffith, W.C., Henderson, R.F., and Brooks, A.L. (1990). Chromosome damage in rat pulmonary alveolar macrophages following ozone inhalation. *Mutat. Res.* **241**, 67-73.

Sarto, F., and Viola, A. (1980). Aberrazioni cromosomiche in soggetti esposti cronicamente ad ozone. *G. Ital. Med. Lav.* **2**, 59-61.

Schwartz, L.W., Dungworth, D.L., Mustafa, M.G., Tarkington, B.K., and Tyler, W.S. (1976). Pulmonary responses of rats to ambient levels of ozone: Effects of 7-day intermittant or continuous exposure. *Lab. Invest.* **34**, 565-578.

Shiraishi, F., and Bandow, H. (1985). The genetic effects of the photochemical reaction products of propylene plus NO_2 on cultured Chinese hamster cells exposed in vitro. *J. Toxicol. Environ. Health* **15**, 531-538.

Speizer, F.E. (1986). Overview of the risk of respiratory cancer from airborne contaminants. *Environ. Health Perspect.* **70**, 9-15.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* **67**, 233-241.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. *Science* **236**, 933-941.

Tice, R.R., Bender, M.A., Ivett, J.L., and Drew, R.T. (1978). Cytogenetic effects of inhaled ozone. *Mutat. Res.* **58**, 293-304.

U.S. Environmental Protection Agency (USEPA) (1986). Air Quality Criteria for Ozone. U.S. EPA, Washington, DC.

Victorin, K. (1992). Review of the genotoxicity of ozone. *Mutat. Res.* **277**, 221-238.

Welsbach Ozone System Corporation (Welsbach) (1980). *Putting Ozone to Work.* Welsbach, Philadelphia.

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

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

Witschi, H.P. (1988). Ozone, nitrogen dioxide and lung cancer: A review of some recent issues and problems. *Toxicology* **48**, 1-20.

Witschi, H., Wilson, D.W., and Plopper, C.G. (1993). Modulation of *N*-nitrosodiethylamine-induced hamster lung tumors by ozone. *Toxicology* **77**, 193-202.

Yokoyama, E., and Frank, R. (1972). Respiratory uptake of ozone in dogs. *Arch. Environ. Health* **25**, 132-138.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four *in vitro* genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 1-14.

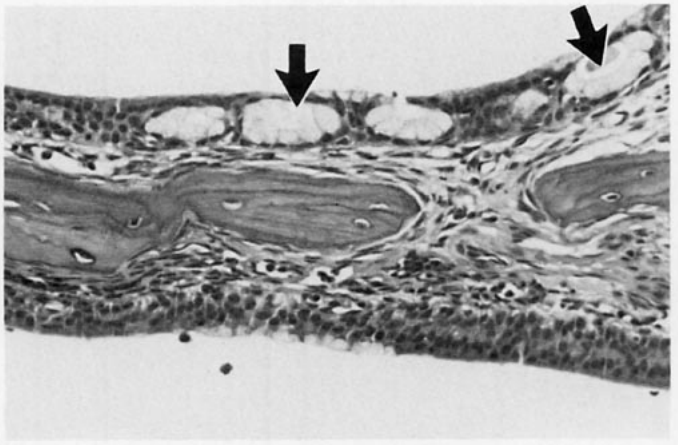


PLATE 1
Clusters of goblet cells (arrows) within the respiratory epithelium of the nasoturbinates in a male F344/N rat exposed to 1.0 ppm ozone for 2 years. H&E; 280×

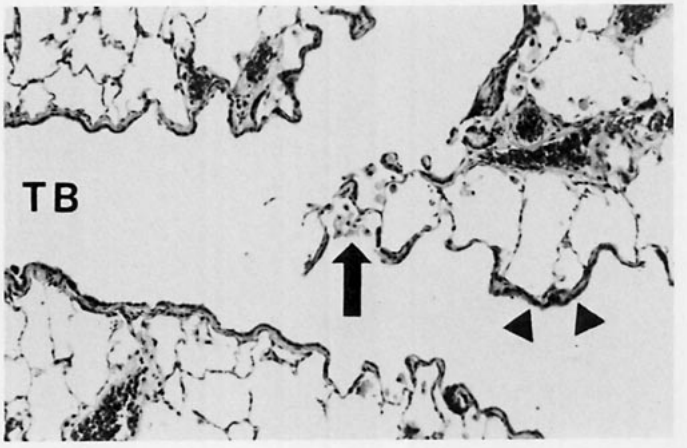


PLATE 2
Centriacinar region of the lung from a female F344/N rat exposed to 1.0 ppm ozone for 2 years. There is a cluster of macrophages (arrow) at the bifurcation of the terminal bronchiole (TB) and also thickening of the epithelium in the alveolar duct (arrowheads). H&E; 120×

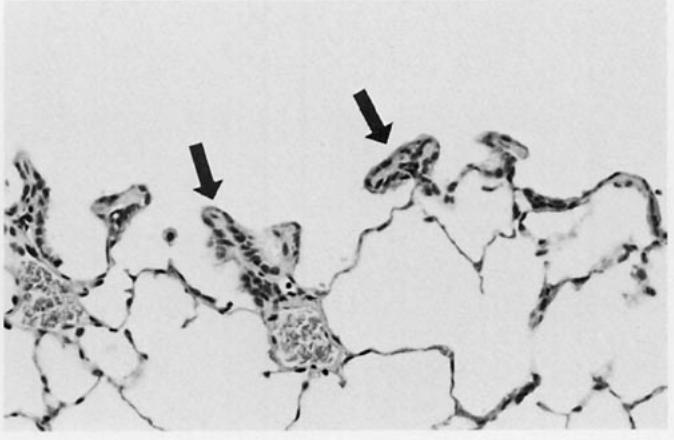


PLATE 3
Cuboidal cells (arrows) occurring between alveoli in the alveolar duct of a female F344/N rat exposed to 1.0 ppm ozone for 2 years. H&E; 210×

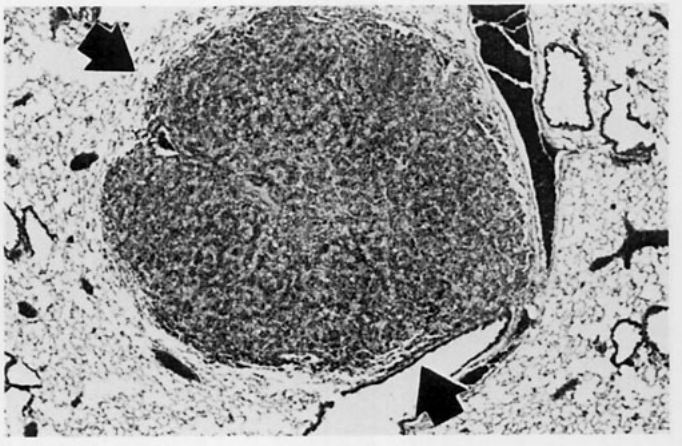


PLATE 4
Alveolar/bronchiolar adenoma from a control male $B6C3F_1$ mouse (arrows). The size and morphology of pulmonary neoplasms was similar in control animals and in animals exposed to ozone. H&E; 28×

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

TABLE A1 Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone 86
TABLE A2 Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone 90
TABLE A3 Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone 94
TABLE A4 Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Male F344/N Rats 100
TABLE A5 Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone 101

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

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1			
Moribund	35	40	36	36
Natural deaths	6	5	7	7
Survivors				
Terminal sacrifice	8	5	7	7
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(49)
Intestine large, cecum	(50)	(50)	(49)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(49)
Intestine small, jejunum	(50)	(50)	(48)	(49)
Intestine small, ileum	(50)	(50)	(49)	(48)
Leiomyoma			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Hepatocellular carcinoma				1 (2%)
Hepatocellular adenoma	2 (4%)	1 (2%)	1 (2%)	
Histiocytic sarcoma			1 (2%)	
Mesentery	(12)	(6)	(12)	(8)
Histiocytic sarcoma			1 (8%)	
Fat, lipoma			1 (8%)	
Oral mucosa	(1)	(1)		
Pharyngeal, squamous cell papilloma	1 (100%)	1 (100%)		
Pancreas	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Histiocytic sarcoma			1 (2%)	
Salivary glands	(49)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(2)	(2)	(1)	(2)
Odontoma				1 (50%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Squamous cell carcinoma, metastatic, lung	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)		1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Endocrine System (continued)				
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Pheochromocytoma complex				1 (2%)
Pheochromocytoma benign	9 (18%)	8 (16%)	16 (32%)	7 (14%)
Bilateral, pheochromocytoma benign	8 (16%)	9 (18%)	8 (16%)	9 (18%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	2 (4%)	5 (10%)	5 (10%)
Adenoma, multiple				1 (2%)
Carcinoma	3 (6%)	3 (6%)	2 (4%)	2 (4%)
Parathyroid gland	(49)	(49)	(48)	(47)
Pituitary gland	(50)	(50)	(49)	(49)
Pars distalis, adenoma	41 (82%)	43 (86%)	42 (86%)	40 (82%)
Pars distalis, carcinoma		1 (2%)		
Thyroid gland	(49)	(50)	(50)	(50)
C-cell, adenoma	1 (2%)	8 (16%)	2 (4%)	1 (2%)
C-cell, carcinoma	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Follicular cell, adenoma		1 (2%)	1 (2%)	1 (2%)
Follicular cell, carcinoma	1 (2%)	1 (2%)		
General Body System				
Peritoneum		(1)		(1)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(49)	(50)	(50)	(49)
Adenoma	3 (6%)		2 (4%)	2 (4%)
Carcinoma	1 (2%)	1 (2%)		1 (2%)
Prostate	(49)	(50)	(50)	(50)
Adenoma			2 (4%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	9 (18%)	14 (28%)	16 (32%)	22 (44%)
Interstitial cell, adenoma	18 (36%)	9 (18%)	15 (30%)	10 (20%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(18)	(10)	(24)	(10)
Renal, histiocytic sarcoma			1 (4%)	
Lymph node, bronchial	(43)	(38)	(44)	(38)
Squamous cell carcinoma, metastatic, lung	1 (2%)			
Lymph node, mandibular	(46)	(46)	(46)	(42)
Lymph node, mesenteric	(49)	(49)	(50)	(50)
Lymph node, mediastinal	(46)	(47)	(48)	(46)
Squamous cell carcinoma, metastatic, lung	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Fibroma	1 (2%)			
Thymus	(44)	(43)	(45)	(41)
Thymoma malignant			1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Integumentary System				
Mammary gland	(33)	(29)	(28)	(30)
Carcinoma			1 (4%)	
Fibroadenoma	2 (6%)	1 (3%)	2 (7%)	1 (3%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)		
Keratoacanthoma	2 (4%)	2 (4%)	2 (4%)	7 (14%)
Keratoacanthoma, multiple	1 (2%)			
Squamous cell carcinoma	1 (2%)	1 (2%)		
Squamous cell papilloma	1 (2%)	1 (2%)		1 (2%)
Trichoepithelioma				1 (2%)
Subcutaneous tissue, fibroma	1 (2%)	4 (8%)	1 (2%)	3 (6%)
Subcutaneous tissue, fibrosarcoma		2 (4%)		
Subcutaneous tissue, hemangioma		1 (2%)		
Subcutaneous tissue, histiocytic sarcoma			1 (2%)	1 (2%)
Subcutaneous tissue, lipoma	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chondrosarcoma	1 (2%)			
Skeletal muscle	(2)		(2)	(1)
Squamous cell carcinoma, metastatic, lung	1 (50%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland		1 (2%)		
Glioma malignant	1 (2%)			
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland			1 (2%)	
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Alveolar/bronchiolar adenoma, multiple				1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Carcinoma, metastatic, thyroid gland			1 (2%)	
Histiocytic sarcoma			1 (2%)	
Osteosarcoma, metastatic, uncertain primary site	1 (2%)			
Pheochromocytoma malignant, metastatic, adrenal medulla				1 (2%)
Squamous cell carcinoma	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Zymbal's gland		(1)	(1)	(1)
Carcinoma			1 (100%)	1 (100%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Lipoma	1 (2%)			
Renal tubule, adenoma	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Renal tubule, adenoma, multiple		1 (2%)		
Renal tubule, carcinoma		1 (2%)		
Transitional epithelium, carcinoma			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, carcinoma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Leukemia mononuclear	27 (54%)	31 (62%)	31 (62%)	27 (54%)
Mesothelioma benign		1 (2%)		2 (4%)
Mesothelioma malignant	2 (4%)		1 (2%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	49	49	49
Total primary neoplasms	152	159	166	159
Total animals with benign neoplasms	48	47	48	49
Total benign neoplasms	111	112	123	119
Total animals with malignant neoplasms	33	35	32	33
Total malignant neoplasms	41	47	43	40
Total animals with metastatic neoplasms	2	1	1	1
Total metastatic neoplasms	5	1	2	1
Total animals with malignant neoplasms uncertain primary site	1			
Total uncertain neoplasms	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 A2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone: 0 ppm

Number of Days on Study	2	3	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6
	4	5	6	8	9	1	1	1	3	4	4	6	6	7	7	8	9	0	0	0	1	2	3	3	3
	7	9	2	1	8	4	6	7	7	3	6	2	5	5	8	3	4	0	1	6	7	4	2	9	9
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	4	0	1	0	0	4	1	5	0	1	2	4	4	4	5	4	3	5	1	4	0	4	2	2
	5	4	9	3	3	2	1	6	4	8	0	6	7	6	2	6	8	5	3	2	5	1	0	0	9
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X																			
Alveolar/bronchiolar carcinoma																									
Osteosarcoma, metastatic, uncertain primary site																							X		
Squamous cell carcinoma				X																					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	
	4	4	4	4	5	7	7	7	7	8	8	8	9	9	0	1	2	3	3	3	3	3	3	3	3	
	3	4	8	8	1	0	0	4	6	1	1	7	5	7	9	5	0	3	3	4	4	4	4	5	5	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Total
	3	2	0	5	1	2	3	2	5	1	3	1	5	2	1	0	3	2	3	2	3	3	3	1	2	Tissues/
	6	5	4	0	7	1	0	2	2	1	9	5	1	7	4	5	2	3	7	8	3	4	8	8	4	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Alveolar/bronchiolar carcinoma																						X				1
Osteosarcoma, metastatic, uncertain primary site																										1
Squamous cell carcinoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

+: Tissue examined microscopically

X: Lesion present

TABLE A2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone: 0.12 ppm

Number of Days on Study	1	4	4	4	4	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6
	7	4	4	6	6	8	8	0	3	3	4	7	9	0	0	0	0	1	1	1	2	2	3	3	4
	5	1	1	6	8	0	9	3	3	7	0	4	1	3	3	5	8	1	6	9	2	5	9	9	2
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	4	1	4	2	2	5	0	2	2	1	4	2	1	0	1	5	3	2	4	1	3	0	1	2	4
	5	0	4	0	1	1	7	4	7	5	7	8	4	8	2	4	3	5	8	1	8	4	8	2	2
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma										X															
Alveolar/bronchiolar carcinoma														X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	
	5	5	6	6	6	7	7	8	8	9	9	9	9	9	0	0	0	1	2	2	3	3	3	3	3	
	3	3	3	7	7	6	8	1	1	1	3	5	5	8	1	9	9	6	3	3	3	4	4	4	5	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Total
	2	3	1	3	3	3	5	1	4	4	0	4	4	5	0	2	3	0	0	1	4	0	1	5	3	Tissues/
	9	6	6	2	7	9	2	3	0	3	1	1	6	3	5	3	0	3	9	7	9	6	9	5	4	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																				X						2
Alveolar/bronchiolar carcinoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone: 0.5 ppm

Number of Days on Study	2	3	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	
	6	8	3	4	5	7	9	9	2	2	2	3	4	7	8	8	9	0	0	1	2	2	2	2	3	
	4	7	3	2	4	0	8	9	0	4	7	9	6	5	3	3	2	4	8	1	1	5	5	5	9	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
	3	4	5	5	3	2	3	4	4	0	3	0	1	4	0	2	2	2	3	3	4	0	1	4	1	
	3	7	5	0	2	0	7	6	0	1	6	4	2	9	6	7	2	8	9	0	3	3	5	5	6	
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Carcinoma, metastatic, thyroid gland									X																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma																										
Alveolar/bronchiolar carcinoma																										
Carcinoma, metastatic, thyroid gland									X																	
Histiocytic sarcoma																								X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	
	4	5	6	6	7	7	8	8	8	9	9	9	0	1	2	2	2	2	3	3	3	3	3	3	3	
	9	4	3	9	1	8	1	1	7	5	7	8	1	5	0	2	3	3	3	3	3	4	4	5	5	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	Total
	5	4	0	3	5	2	0	3	3	1	5	4	4	4	0	2	1	1	1	1	2	1	5	2	3	Tissues/
	4	8	8	1	1	6	9	4	8	8	2	4	1	2	5	4	1	3	0	9	5	7	3	1	5	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland																										1
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma												X								X						2
Alveolar/bronchiolar carcinoma																							X			1
Carcinoma, metastatic, thyroid gland																										1
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone: 1.0 ppm

Number of Days on Study	3	4	4	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6
	7	2	5	0	2	4	5	5	6	6	7	8	8	9	9	0	1	1	1	1	1	2	2	2	3
	2	2	8	2	6	0	2	9	2	8	4	3	3	0	6	0	1	5	6	6	9	1	3	3	5
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	4	4	2	3	3	0	2	5	1	3	1	1	2	5	4	1	2	4	0	1	2	2	3	4	3
	8	4	4	1	5	4	6	5	0	0	4	8	5	4	7	5	1	9	1	9	7	8	8	2	4
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar adenoma, multiple																					X				
Alveolar/bronchiolar carcinoma																									
Pheochromocytoma malignant, metastatic, adrenal medulla																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	
	4	4	5	5	5	5	5	5	6	6	6	6	8	9	0	1	2	2	3	3	3	3	3	3	3	
	0	4	1	3	4	8	8	9	5	7	7	7	1	5	9	5	3	3	3	4	4	5	5	5	5	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	Total
	1	5	2	1	3	0	2	2	5	1	4	4	0	3	3	0	0	5	5	0	4	0	1	1	4	Tissues/
	2	3	0	7	9	5	3	2	6	6	0	1	8	3	6	9	6	1	2	7	5	3	1	3	3	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																X						X				2
Alveolar/bronchiolar adenoma, multiple																										1
Alveolar/bronchiolar carcinoma										X																1
Pheochromocytoma malignant, metastatic, adrenal medulla																X										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	17/50 (34%)	17/50 (34%)	24/50 (48%)	16/50 (32%)
Adjusted rate[b]	83.0%	80.5%	94.2%	81.6%
Terminal rate[c]	5/8 (63%)	2/5 (40%)	6/7 (86%)	4/7 (57%)
First incidence (days)	606	605	442	526
Life table test[d]	P=0.506	P=0.498	P=0.191	P=0.549
Logistic regression test[d]	P=0.527	P=0.530N	P=0.092	P=0.514N
Cochran-Armitage test[d]	P=0.527			
Fisher exact test[d]		P=0.583N	P=0.111	P=0.500N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	17/50 (34%)	18/50 (36%)	25/50 (50%)	18/50 (36%)
Adjusted rate	83.0%	81.4%	94.9%	83.9%
Terminal rate	5/8 (63%)	2/5 (40%)	6/7 (86%)	4/7 (57%)
First incidence (days)	606	605	442	526
Life table test	P=0.383	P=0.430	P=0.153	P=0.405
Logistic regression test	P=0.365	P=0.570	P=0.059	P=0.495
Cochran-Armitage test	P=0.380			
Fisher exact test		P=0.500	P=0.078	P=0.500
Kidney (Renal Tubule): Adenoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	18.0%	38.3%	22.9%	8.3%
Terminal rate	1/8 (13%)	1/5 (20%)	1/7 (14%)	0/7 (0%)
First incidence (days)	681	709	722	695
Life table test	P=0.266N	P=0.406	P=0.684N	P=0.548N
Logistic regression test	P=0.275N	P=0.474	P=0.667N	P=0.514N
Cochran-Armitage test	P=0.280N			
Fisher exact test		P=0.500	P=0.691N	P=0.500N
Kidney (Renal Tubule): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	18.0%	38.3%	22.9%	8.3%
Terminal rate	1/8 (13%)	1/5 (20%)	1/7 (14%)	0/7 (0%)
First incidence (days)	681	709	722	695
Life table test	P=0.266N	P=0.406	P=0.684N	P=0.548N
Logistic regression test	P=0.275N	P=0.474	P=0.667N	P=0.514N
Cochran-Armitage test	P=0.280N			
Fisher exact test		P=0.500	P=0.691N	P=0.500N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/50 (2%)	2/50 (4%)	2/50 (4%)	3/50 (6%)
Adjusted rate	2.2%	16.4%	20.4%	25.4%
Terminal rate	0/8 (0%)	0/5 (0%)	1/7 (14%)	1/7 (14%)
First incidence (days)	514	537	698	619
Life table test	P=0.271	P=0.474	P=0.504	P=0.302
Logistic regression test	P=0.246	P=0.500	P=0.501	P=0.309
Cochran-Armitage test	P=0.244			
Fisher exact test		P=0.500	P=0.500	P=0.309

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	4/50 (8%)
Adjusted rate	14.4%	18.6%	33.7%	30.1%
Terminal rate	1/8 (13%)	0/5 (0%)	2/7 (29%)	1/7 (14%)
First incidence (days)	514	537	698	619
Life table test	P=0.301	P=0.454	P=0.479	P=0.307
Logistic regression test	P=0.284	P=0.500	P=0.515	P=0.341
Cochran-Armitage test	P=0.283			
Fisher exact test		P=0.500	P=0.500	P=0.339
Pancreatic Islets: Adenoma				
Overall rate	4/50 (8%)	2/50 (4%)	5/50 (10%)	6/50 (12%)
Adjusted rate	15.1%	6.1%	49.8%	35.0%
Terminal rate	0/8 (0%)	0/5 (0%)	3/7 (43%)	1/7 (14%)
First incidence (days)	578	591	625	621
Life table test	P=0.156	P=0.312N	P=0.498	P=0.370
Logistic regression test	P=0.148	P=0.337N	P=0.511	P=0.378
Cochran-Armitage test	P=0.148			
Fisher exact test		P=0.339N	P=0.500	P=0.370
Pancreatic Islets: Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	17.8%	27.7%	21.4%	18.6%
Terminal rate	1/8 (13%)	1/5 (20%)	1/7 (14%)	1/7 (14%)
First incidence (days)	565	639	715	658
Life table test	P=0.358N	P=0.611	P=0.517N	P=0.524N
Logistic regression test	P=0.359N	P=0.656N	P=0.489N	P=0.495N
Cochran-Armitage test	P=0.357N			
Fisher exact test		P=0.661N	P=0.500N	P=0.500N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	5/50 (10%)	7/50 (14%)	8/50 (16%)
Adjusted rate	30.2%	32.2%	65.5%	48.5%
Terminal rate	1/8 (13%)	1/5 (20%)	4/7 (57%)	2/7 (29%)
First incidence (days)	565	591	625	621
Life table test	P=0.319	P=0.399N	P=0.600	P=0.481
Logistic regression test	P=0.311	P=0.374N	P=0.602N	P=0.512
Cochran-Armitage test	P=0.314			
Fisher exact test		P=0.380N	P=0.613N	P=0.500
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	41/50 (82%)	43/50 (86%)	42/49 (86%)	40/49 (82%)
Adjusted rate	94.3%	100.0%	100.0%	95.1%
Terminal rate	6/8 (75%)	5/5 (100%)	7/7 (100%)	5/7 (71%)
First incidence (days)	462	441	387	422
Life table test	P=0.464N	P=0.412	P=0.544N	P=0.531
Logistic regression test	P=0.378N	P=0.392	P=0.363	P=0.487N
Cochran-Armitage test	P=0.452N			
Fisher exact test		P=0.393	P=0.410	P=0.584N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	41/50 (82%)	44/50 (88%)	42/49 (86%)	40/49 (82%)
Adjusted rate	94.3%	100.0%	100.0%	95.1%
Terminal rate	6/8 (75%)	5/5 (100%)	7/7 (100%)	5/7 (71%)
First incidence (days)	462	441	387	422
Life table test	P=0.439N	P=0.369	P=0.544N	P=0.531
Logistic regression test	P=0.323N	P=0.282	P=0.363	P=0.487N
Cochran-Armitage test	P=0.396N			
Fisher exact test		P=0.288	P=0.410	P=0.584N
Preputial Gland: Adenoma				
Overall rate	3/49 (6%)	0/50 (0%)	2/50 (4%)	2/49 (4%)
Adjusted rate	24.5%	0.0%	18.4%	4.1%
Terminal rate	1/8 (13%)	0/5 (0%)	1/7 (14%)	0/7 (0%)
First incidence (days)	676	–[e]	671	372
Life table test	P=0.509	P=0.140N	P=0.475N	P=0.532N
Logistic regression test	P=0.533	P=0.113N	P=0.462N	P=0.514N
Cochran-Armitage test	P=0.531			
Fisher exact test		P=0.117N	P=0.490N	P=0.500N
Preputial Gland: Adenoma or Carcinoma				
Overall rate	4/49 (8%)	1/50 (2%)	2/50 (4%)	3/49 (6%)
Adjusted rate	26.2%	3.6%	18.4%	8.5%
Terminal rate	1/8 (13%)	0/5 (0%)	1/7 (14%)	0/7 (0%)
First incidence (days)	498	639	671	372
Life table test	P=0.536	P=0.205N	P=0.322N	P=0.518N
Logistic regression test	P=0.556	P=0.170N	P=0.320N	P=0.523N
Cochran-Armitage test	P=0.557			
Fisher exact test		P=0.175N	P=0.329N	P=0.500N
Skin: Keratoacanthoma				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	7/50 (14%)
Adjusted rate	37.5%	7.5%	10.5%	39.0%
Terminal rate	3/8 (38%)	0/5 (0%)	0/7 (0%)	1/7 (14%)
First incidence (days)	733 (T)	591	669	526
Life table test	P=0.042	P=0.605N	P=0.509N	P=0.131
Logistic regression test	P=0.048	P=0.492N	P=0.457N	P=0.152
Cochran-Armitage test	P=0.050			
Fisher exact test		P=0.500N	P=0.500N	P=0.159
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Squamous Cell Carcinoma				
Overall rate	3/50 (6%)	4/50 (8%)	2/50 (4%)	9/50 (18%)
Adjusted rate	37.5%	33.4%	10.5%	50.7%
Terminal rate	3/8 (38%)	1/5 (20%)	0/7 (0%)	1/7 (14%)
First incidence (days)	733 (T)	591	669	526
Life table test	P=0.030	P=0.365	P=0.509N	P=0.055
Logistic regression test	P=0.027	P=0.506	P=0.457N	P=0.053
Cochran-Armitage	P=0.031			
Fisher exact test		P=0.500	P=0.500N	P=0.061

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)	3/50 (6%)
Adjusted rate	10.0%	40.6%	11.1%	22.4%
Terminal rate	0/8 (0%)	1/5 (20%)	0/7 (0%)	1/7 (14%)
First incidence (days)	715	681	723	621
Life table test	P=0.478	P=0.149	P=0.727N	P=0.278
Logistic regression test	P=0.457	P=0.162	P=0.746N	P=0.299
Cochran-Armitage test	P=0.470			
Fisher exact test		P=0.181	P=0.753N	P=0.309
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Histiocytic Sarcoma				
Overall rate	1/50 (2%)	6/50 (12%)	1/50 (2%)	3/50 (6%)
Adjusted rate	10.0%	43.6%	11.1%	22.4%
Terminal rate	0/8 (0%)	1/5 (20%)	0/7 (0%)	1/7 (14%)
First incidence (days)	715	489	723	621
Life table test	P=0.495N	P=0.053	P=0.727N	P=0.278
Logistic regression test	P=0.504N	P=0.060	P=0.746N	P=0.299
Cochran-Armitage test	P=0.505N			
Fisher exact test		P=0.056	P=0.753N	P=0.309
Testes: Adenoma				
Overall rate	27/50 (54%)	23/50 (46%)	31/50 (62%)	32/50 (64%)
Adjusted rate	100.0%	84.1%	88.9%	95.9%
Terminal rate	8/8 (100%)	2/5 (40%)	4/7 (57%)	6/7 (86%)
First incidence (days)	462	537	442	372
Life table test	P=0.128	P=0.402N	P=0.383	P=0.220
Logistic regression test	P=0.065	P=0.226N	P=0.252	P=0.235
Cochran-Armitage test	P=0.060			
Fisher exact test		P=0.274N	P=0.272	P=0.208
Thyroid Gland (C-cell): Adenoma				
Overall rate	1/49 (2%)	8/50 (16%)	2/50 (4%)	1/50 (2%)
Adjusted rate	2.4%	44.4%	15.2%	2.6%
Terminal rate	0/8 (0%)	1/5 (20%)	0/7 (0%)	0/7 (0%)
First incidence (days)	543	441	697	583
Life table test	P=0.105N	P=0.022	P=0.540	P=0.740N
Logistic regression test	P=0.100N	P=0.019	P=0.510	P=0.744
Cochran-Armitage test	P=0.100N			
Fisher exact test		P=0.017	P=0.508	P=0.747N
Thyroid Gland (C-cell): Carcinoma				
Overall rate	1/49 (2%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	5.6%	16.7%	16.3%	3.4%
Terminal rate	0/8 (0%)	0/5 (0%)	1/7 (14%)	0/7 (0%)
First incidence (days)	674	653	520	621
Life table test	P=0.434N	P=0.346	P=0.497	P=0.725
Logistic regression test	P=0.402N	P=0.320	P=0.508	P=0.755N
Cochran-Armitage test	P=0.407N			
Fisher exact test		P=0.316	P=0.508	P=0.747N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	2/49 (4%)	11/50 (22%)	4/50 (8%)	2/50 (4%)
Adjusted rate	7.9%	53.7%	29.0%	5.9%
Terminal rate	0/8 (0%)	1/5 (20%)	1/7 (14%)	0/7 (0%)
First incidence (days)	543	441	520	583
Life table test	P=0.106N	P=0.015	P=0.365	P=0.679
Logistic regression test	P=0.088N	P=0.010	P=0.348	P=0.694
Cochran-Armitage test	P=0.090N			
Fisher exact test		P=0.008	P=0.349	P=0.684N
All Organs: Mononuclear Cell Leukemia				
Overall rate	27/50 (54%)	31/50 (62%)	31/50 (62%)	27/50 (54%)
Adjusted rate	94.9%	95.4%	95.1%	79.9%
Terminal rate	7/8 (88%)	4/5 (80%)	6/7 (86%)	3/7 (43%)
First incidence (days)	514	441	264	502
Life table test	P=0.449N	P=0.235	P=0.346	P=0.515
Logistic regression test	P=0.406N	P=0.276	P=0.265	P=0.554N
Cochran-Armitage test	P=0.426N			
Fisher exact test		P=0.272	P=0.272	P=0.579N
All Organs: Benign Mesothelioma				
Overall rate	2/50 (4%)	1/50 (2%)	1/50 (2%)	5/50 (10%)
Adjusted rate	11.0%	2.6%	5.0%	22.2%
Terminal rate	0/8 (0%)	0/5 (0%)	0/7 (0%)	0/7 (0%)
First incidence (days)	462	574	678	583
Life table test	P=0.067	P=0.507N	P=0.472N	P=0.227
Logistic regression test	P=0.065	P=0.499N	P=0.500N	P=0.214
Cochran-Armitage test	P=0.066			
Fisher exact test		P=0.500N	P=0.500N	P=0.218
All Organs: Benign Neoplasms				
Overall rate	48/50 (96%)	47/50 (94%)	48/50 (96%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	8/8 (100%)	5/5 (100%)	7/7 (100%)	7/7 (100%)
First incidence (days)	462	441	387	372
Life table test	P=0.430	P=0.529	P=0.487N	P=0.440
Logistic regression test	P=0.431	P=0.467N	P=0.731	P=0.806
Cochran-Armitage test	P=0.280			
Fisher exact test		P=0.500N	P=0.691N	P=0.500
All Organs: Malignant Neoplasms				
Overall rate	33/50 (66%)	35/50 (70%)	32/50 (64%)	33/50 (66%)
Adjusted rate	95.7%	96.1%	95.7%	92.3%
Terminal rate	7/8 (88%)	4/5 (80%)	6/7 (86%)	5/7 (71%)
First incidence (days)	359	441	264	502
Life table test	P=0.464N	P=0.361	P=0.482N	P=0.497
Logistic regression test	P=0.417N	P=0.418	P=0.502N	P=0.567N
Cochran-Armitage test	P=0.437N			
Fisher exact test		P=0.415	P=0.500N	P=0.583N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	49/50 (98%)	49/50 (98%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	8/8 (100%)	5/5 (100%)	7/7 (100%)	7/7 (100%)
First incidence (days)	359	441	264	372
Life table test	P=0.503	P=0.483	P=0.487N	P=0.485
Logistic regression test	P=0.410N	P=0.638N	P=0.777	P=0.555N
Cochran-Armitage test	P=0.627			
Fisher exact test		P=0.753N	P=0.753N	P=0.753N

(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, pancreas, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE A4
Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Northwest			
o-Chlorobenzalmalononitrile	4/50	0/50	4/50
α-Chloroacetophenone	1/49	1/49	2/49
Epinephrine hydrochloride	4/50	1/50	5/50
Ethyl chloride	0/50	0/50	0/50
Hexachlorocyclopentadiene	5/50	0/50	5/50
Overall Historical Incidence			
Total	15/398 (3.8%)	2/398 (0.5%)	17/398 (4.3%)
Standard deviation	4.2%	0.9%	4.5%
Range	0%-10%	0%-2%	0%-10%

[a] Data as of 31 March 1993

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone[a]

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	1			
Moribund	35	40	36	36
Natural deaths	6	5	7	7
Survivors				
Terminal sacrifice	8	5	7	7
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)			
Mineralization	1 (2%)	1 (2%)		
Parasite metazoan	3 (6%)	4 (8%)	9 (18%)	6 (12%)
Intestine large, rectum	(50)	(50)	(50)	(49)
Mineralization		1 (2%)		
Parasite metazoan	2 (4%)	2 (4%)	2 (4%)	3 (6%)
Intestine large, cecum	(50)	(50)	(49)	(50)
Inflammation, chronic active	1 (2%)			
Parasite metazoan	3 (6%)	5 (10%)	3 (6%)	5 (10%)
Intestine small, duodenum	(50)	(50)	(50)	(49)
Hyperplasia, adenomatous	1 (2%)			
Necrosis	1 (2%)	3 (6%)		1 (2%)
Intestine small, ileum	(50)	(50)	(49)	(48)
Inflammation, acute		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	4 (8%)	1 (2%)	2 (4%)	4 (8%)
Basophilic focus	14 (28%)	18 (36%)	11 (22%)	9 (18%)
Clear cell focus	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Degeneration, cystic	13 (26%)	16 (32%)	19 (38%)	14 (28%)
Degeneration, fatty	9 (18%)	5 (10%)	5 (10%)	4 (8%)
Eosinophilic focus	1 (2%)	4 (8%)	2 (4%)	2 (4%)
Fibrosis	1 (2%)			
Hepatodiaphragmatic nodule	3 (6%)	5 (10%)	2 (4%)	4 (8%)
Inflammation, granulomatous	2 (4%)			
Mineralization	1 (2%)			
Mixed cell focus	2 (4%)	1 (2%)	4 (8%)	1 (2%)
Necrosis	2 (4%)	3 (6%)	1 (2%)	
Regeneration	2 (4%)			2 (4%)
Thrombosis	1 (2%)		1 (2%)	
Bile duct, hyperplasia	29 (58%)	34 (68%)	37 (74%)	33 (66%)
Centrilobular, necrosis	3 (6%)	6 (12%)	3 (6%)	2 (4%)
Mesentery	(12)	(6)	(12)	(8)
Thrombosis	1 (8%)			
Artery, inflammation, chronic active	1 (8%)		1 (8%)	
Artery, mineralization	3 (25%)	2 (33%)	1 (8%)	1 (13%)
Fat, necrosis	7 (58%)	4 (67%)	9 (75%)	5 (63%)

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

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Atrophy	22 (44%)	23 (46%)	30 (60%)	26 (52%)
Basophilic focus	1 (2%)		4 (8%)	
Hyperplasia	2 (4%)	4 (8%)	4 (8%)	2 (4%)
Thrombosis		1 (2%)		
Artery, inflammation	3 (6%)	1 (2%)	1 (2%)	
Artery, mineralization	2 (4%)			
Salivary glands	(49)	(50)	(50)	(50)
Inflammation, chronic			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum			2 (4%)	
Foreign body				1 (2%)
Hyperplasia, squamous	4 (8%)	2 (4%)	1 (2%)	3 (6%)
Inflammation, acute	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Mineralization	2 (4%)	5 (10%)	1 (2%)	4 (8%)
Necrosis	4 (8%)	7 (14%)	9 (18%)	7 (14%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Inflammation, acute	1 (2%)	1 (2%)		1 (2%)
Mineralization	6 (12%)	7 (14%)	7 (14%)	10 (20%)
Necrosis	2 (4%)	4 (8%)	3 (6%)	3 (6%)
Tooth	(2)	(2)	(1)	(2)
Developmental malformation	2 (100%)	2 (100%)		
Inflammation, chronic active			1 (100%)	1 (50%)
Cardiovascular System				
Blood vessel	(4)	(4)	(1)	(2)
Aorta, mineralization	4 (100%)	4 (100%)	1 (100%)	2 (100%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	39 (78%)	37 (74%)	44 (88%)	36 (72%)
Mineralization	1 (2%)	2 (4%)		
Thrombosis		2 (4%)		
Artery, mineralization	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Atrium, thrombosis	2 (4%)	3 (6%)	5 (10%)	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule		1 (2%)		
Hyperplasia	24 (48%)	20 (40%)	23 (46%)	18 (36%)
Hypertrophy	6 (12%)	7 (14%)	7 (14%)	7 (14%)
Necrosis	2 (4%)		2 (4%)	2 (4%)
Vacuolization cytoplasmic	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	23 (46%)	29 (58%)	24 (48%)	17 (34%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	1 (2%)	2 (4%)	
Parathyroid gland	(49)	(49)	(48)	(47)
Hyperplasia	10 (20%)	10 (20%)	15 (31%)	11 (23%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Endocrine System (continued)				
Pituitary gland	(50)	(50)	(49)	(49)
Cyst	1 (2%)			
Mineralization		2 (4%)	1 (2%)	
Pars distalis, hemorrhage				1 (2%)
Pars distalis, hyperplasia		3 (6%)	3 (6%)	5 (10%)
Pars distalis, metaplasia, osseous	1 (2%)			
Pars intermedia, hyperplasia		1 (2%)		
Thyroid gland	(49)	(50)	(50)	(50)
C-cell, hyperplasia	29 (59%)	25 (50%)	31 (62%)	16 (32%)
Follicular cell, hyperplasia	2 (4%)	1 (2%)	2 (4%)	2 (4%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Preputial gland	(49)	(50)	(50)	(49)
Cyst			1 (2%)	
Inflammation, chronic active	4 (8%)	2 (4%)	3 (6%)	6 (12%)
Prostate	(49)	(50)	(50)	(50)
Hyperplasia	1 (2%)		1 (2%)	1 (2%)
Inflammation, chronic active	7 (14%)	7 (14%)	5 (10%)	6 (12%)
Inflammation, suppurative	2 (4%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)		1 (2%)	
Mineralization		1 (2%)		
Testes	(50)	(50)	(50)	(50)
Atrophy	10 (20%)	4 (8%)	6 (12%)	6 (12%)
Artery, inflammation, chronic active	7 (14%)	8 (16%)	4 (8%)	3 (6%)
Interstitial cell, hyperplasia	13 (26%)	13 (26%)	13 (26%)	9 (18%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Lymph node	(18)	(10)	(24)	(10)
Iliac, hemorrhage	1 (6%)			
Renal, angiectasis	1 (6%)			
Renal, hemorrhage	6 (33%)	2 (20%)	6 (25%)	1 (10%)
Renal, inflammation, granulomatous			1 (4%)	
Lymph node, bronchial	(43)	(38)	(44)	(38)
Fibrosis				1 (3%)
Hemorrhage	1 (2%)			
Lymph node, mandibular	(46)	(46)	(46)	(42)
Hemorrhage	1 (2%)	1 (2%)		
Infiltration cellular, plasma cell	1 (2%)	2 (4%)	2 (4%)	2 (5%)
Lymph node, mesenteric	(49)	(49)	(50)	(50)
Ectasia		1 (2%)		
Hemorrhage	1 (2%)			

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Hematopoietic System (continued)				
Lymph node, mediastinal	(46)	(47)	(48)	(46)
Hemorrhage		1 (2%)	1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Fibrosis	12 (24%)	11 (22%)	16 (32%)	14 (28%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)	1 (2%)	
Hemorrhage			1 (2%)	
Hyperplasia, focal				1 (2%)
Necrosis	2 (4%)	5 (10%)	1 (2%)	1 (2%)
Integumentary System				
Mammary gland	(33)	(29)	(28)	(30)
Galactocele	2 (6%)	1 (3%)	1 (4%)	1 (3%)
Skin	(50)	(50)	(50)	(50)
Hyperkeratosis	3 (6%)	3 (6%)	1 (2%)	
Inflammation, chronic active	2 (4%)	8 (16%)	2 (4%)	1 (2%)
Prepuce, inflammation, acute	3 (6%)	4 (8%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy	7 (14%)	8 (16%)	8 (16%)	4 (8%)
Hyperostosis		1 (2%)		1 (2%)
Skeletal muscle	(2)		(2)	(1)
Hemorrhage	1 (50%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	2 (4%)			
Necrosis	1 (2%)			1 (2%)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Inflammation, acute	1 (2%)			
Mineralization	1 (2%)			1 (2%)
Epiglottis, metaplasia, squamous		2 (4%)	16 (32%)	43 (86%)
Lung	(50)	(50)	(50)	(50)
Congestion, chronic			1 (2%)	
Hemorrhage	2 (4%)	2 (4%)		
Inflammation, chronic active	2 (4%)			1 (2%)
Inflammation, suppurative		1 (2%)		
Metaplasia, osseous	2 (4%)	1 (2%)	1 (2%)	
Mineralization	3 (6%)	4 (8%)	1 (2%)	2 (4%)
Thrombosis	1 (2%)	1 (2%)		
Alveolar epithelium, hyperplasia	6 (12%)	6 (12%)	3 (6%)	4 (8%)
Alveolar epithelium, metaplasia		9 (18%)	46 (92%)	47 (94%)
Alveolus, edema	1 (2%)			1 (2%)
Alveolus, infiltration cellular, histiocyte	1 (2%)		27 (54%)	42 (84%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Respiratory System (continued)				
Lung (continued)	(50)	(50)	(50)	(50)
Artery, infiltration cellular, histiocyte	1 (2%)			
Artery, mediastinum, inflammation	1 (2%)			
Artery, mediastinum, mineralization	5 (10%)	3 (6%)	1 (2%)	2 (4%)
Bronchiole, necrosis				1 (2%)
Interstitium, fibrosis		2 (4%)	40 (80%)	44 (88%)
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	3 (6%)	10 (20%)	12 (24%)	20 (40%)
Thrombosis	8 (16%)	13 (26%)	12 (24%)	8 (16%)
Goblet cell, lateral wall, hyperplasia	1 (2%)	4 (8%)	41 (82%)	48 (96%)
Lateral wall, hyperplasia		8 (16%)	50 (100%)	49 (98%)
Lateral wall, metaplasia, squamous	2 (4%)	6 (12%)	36 (72%)	46 (92%)
Olfactory epithelium, degeneration, hyaline	50 (100%)	50 (100%)	50 (100%)	50 (100%)
Olfactory epithelium, metaplasia	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Trachea	(50)	(50)	(50)	(50)
Inflammation, acute		1 (2%)	1 (2%)	
Metaplasia, squamous	1 (2%)			1 (2%)
Mineralization	2 (4%)	1 (2%)		2 (4%)
Special Senses System				
Eye	(3)	(1)	(2)	
Cataract	2 (67%)		2 (100%)	
Degeneration		1 (100%)		
Hemorrhage	1 (33%)			
Cornea, inflammation, chronic active			1 (50%)	
Cornea, mineralization	1 (33%)			
Retina, atrophy	2 (67%)		1 (50%)	
Zymbal's gland		(1)	(1)	(1)
Hyperplasia, squamous		1 (100%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Cyst	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Infarct	1 (2%)	2 (4%)		1 (2%)
Mineralization	4 (8%)	5 (10%)	1 (2%)	2 (4%)
Nephropathy	49 (98%)	48 (96%)	50 (100%)	50 (100%)
Thrombosis	1 (2%)		1 (2%)	
Artery, inflammation	1 (2%)		1 (2%)	
Papilla, necrosis	1 (2%)			
Pelvis, inflammation, acute	2 (4%)	3 (6%)	4 (8%)	2 (4%)
Pelvis, transitional epithelium, hyperplasia	1 (2%)			
Renal tubule, hyperplasia	1 (2%)	3 (6%)	1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Inflammation, acute	2 (4%)			
Inflammation, chronic active	1 (2%)	3 (6%)	4 (8%)	1 (2%)
Transitional epithelium, hyperplasia	1 (2%)	1 (2%)		1 (2%)

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

TABLE B1 Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Ozone 108
TABLE B2 Individual Animal Respiratory System Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Ozone 112
TABLE B3 Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Ozone 116
TABLE B4 Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Female F344/N Rats 120
TABLE B5 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Ozone 121

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

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	19	22	17	16
Natural deaths	3	4	3	7
Survivors				
Terminal sacrifice	28	24	30	27
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(50)	(50)	(50)	(49)
Intestine large, rectum	(43)	(48)	(45)	(48)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(49)	(49)	(48)	(47)
Intestine small, ileum	(50)	(49)	(48)	(47)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma		1 (2%)		
Histiocytic sarcoma	1 (2%)		1 (2%)	1 (2%)
Mesentery	(4)	(5)	(11)	(4)
Sarcoma			1 (9%)	
Oral mucosa	(1)	(1)		
Pharyngeal, squamous cell papilloma		1 (100%)		
Pancreas	(50)	(50)	(50)	(49)
Salivary glands	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)	(1)		(1)
Squamous cell papilloma	1 (100%)	1 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	2 (4%)	1 (2%)
Carcinoma	1 (2%)	1 (2%)		
Histiocytic sarcoma	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Ganglioneuroma				1 (2%)
Pheochromocytoma complex		1 (2%)		
Pheochromocytoma benign	5 (10%)	5 (10%)	5 (10%)	4 (8%)
Bilateral, pheochromocytoma benign	1 (2%)		1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(49)
Adenoma		1 (2%)		
Carcinoma	1 (2%)	1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Endocrine System (continued)				
Pituitary gland	(50)	(49)	(50)	(49)
Histiocytic sarcoma	1 (2%)			
Pars distalis, adenoma	34 (68%)	36 (73%)	38 (76%)	33 (67%)
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma	1 (2%)			1 (2%)
C-cell, adenoma	4 (8%)	5 (10%)	5 (10%)	1 (2%)
C-cell, carcinoma		1 (2%)	2 (4%)	
Follicular cell, adenoma			1 (2%)	
Follicular cell, carcinoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(43)	(50)	(47)	(47)
Adenoma	5 (12%)	3 (6%)	7 (15%)	8 (17%)
Carcinoma		3 (6%)	3 (6%)	1 (2%)
Histiocytic sarcoma			1 (2%)	
Bilateral, adenoma			1 (2%)	
Ovary	(50)	(50)	(49)	(49)
Arrhenoblastoma malignant	1 (2%)			
Granulosa cell tumor malignant		1 (2%)	1 (2%)	
Granulosa cell tumor benign	1 (2%)			
Granulosa-theca tumor malignant		1 (2%)		
Histiocytic sarcoma	1 (2%)			
Uterus	(50)	(50)	(49)	(50)
Deciduoma benign				1 (2%)
Polyp stromal	8 (16%)	11 (22%)	7 (14%)	6 (12%)
Polyp stromal, multiple	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Sarcoma stromal, multiple	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)		1 (2%)	
Lymph node	(5)	(9)	(3)	(3)
Carcinoma, metastatic, thyroid gland		1 (11%)		
Iliac, histiocytic sarcoma	1 (20%)			
Pancreatic, histiocytic sarcoma	1 (20%)			
Lymph node, bronchial	(43)	(39)	(36)	(43)
Histiocytic sarcoma	1 (2%)			
Osteosarcoma, metastatic, bone		1 (3%)		
Lymph node, mandibular	(48)	(47)	(46)	(46)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Histiocytic sarcoma	1 (2%)			
Lymph node, mesenteric	(49)	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Hematopoietic System (continued)				
Lymph node, mediastinal	(39)	(46)	(41)	(47)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Histiocytic sarcoma	1 (3%)		1 (2%)	
Osteosarcoma, metastatic, bone		1 (2%)		
Spleen	(50)	(50)	(50)	(49)
Histiocytic sarcoma	1 (2%)			
Thymus	(45)	(43)	(46)	(49)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Histiocytic sarcoma	1 (2%)			
Thymoma malignant				1 (2%)
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Adenoma	1 (2%)	1 (2%)	1 (2%)	
Carcinoma	4 (8%)	1 (2%)	3 (6%)	1 (2%)
Fibroadenoma	18 (36%)	12 (24%)	22 (44%)	8 (16%)
Fibroadenoma, multiple	2 (4%)	5 (10%)	1 (2%)	4 (8%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma				1 (2%)
Keratoacanthoma		1 (2%)		
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma	1 (2%)			1 (2%)
Subcutaneous tissue, fibroma	1 (2%)			
Subcutaneous tissue, histiocytic sarcoma			1 (2%)	
Subcutaneous tissue, lipoma		1 (2%)	1 (2%)	
Subcutaneous tissue, melanoma malignant		1 (2%)		
Subcutaneous tissue, schwannoma malignant	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma		1 (2%)		
Skeletal muscle	(1)			(3)
Rhabdomyosarcoma				1 (33%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma benign				1 (2%)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma			2 (4%)	
Carcinoma, metastatic, mammary gland				1 (2%)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Carcinoma, metastatic, adrenal cortex		1 (2%)		
Histiocytic sarcoma	1 (2%)		1 (2%)	
Osteosarcoma, metastatic, bone		1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Respiratory System (continued)				
Nose	(50)	(50)	(50)	(50)
Glands, adenoma			1 (2%)	
Special Senses System				
Zymbal's gland				(1)
Carcinoma				1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Renal tubule, adenoma	1 (2%)	1 (2%)		
Urinary bladder	(50)	(49)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)		1 (2%)	1 (2%)
Leukemia mononuclear	17 (34%)	18 (36%)	16 (32%)	17 (34%)
Mesothelioma malignant			1 (2%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	48	48	46
Total primary neoplasms	116	118	125	97
Total animals with benign neoplasms	41	43	45	41
Total benign neoplasms	86	87	96	72
Total animals with malignant neoplasms	26	28	26	23
Total malignant neoplasms	30	31	29	25
Total animals with metastatic neoplasms		3		1
Total metastatic neoplasms		10		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 B2
Individual Animal Respiratory System Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Ozone: 0 ppm

Number of Days on Study	3	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	
	6	4	4	0	2	3	5	6	7	0	0	3	6	6	8	8	8	9	0	0	0	2	3	3	3	
	2	3	6	2	7	7	3	6	1	3	8	0	5	7	1	7	8	5	9	9	9	3	3	3	3	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	5	5	3	2	1	1	3	0	3	1	3	4	4	0	2	1	4	2	0	1	3	2	0	1	1	
	3	6	3	5	0	1	6	6	4	9	5	1	6	2	4	3	5	2	8	6	0	9	9	4	7	
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Histiocytic sarcoma				X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Total
	1	2	2	2	3	4	4	5	0	0	0	2	3	4	5	0	1	2	2	3	3	3	4	4	5	Tissues/
	8	0	7	8	9	3	4	2	1	4	5	3	1	8	0	7	2	1	6	2	7	8	2	7	1	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

+: Tissue examined microscopically

X: Lesion present

TABLE B2
Individual Animal Respiratory System Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Ozone: 0.12 ppm

Number of Days on Study	2	4	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	
	7	1	6	7	4	6	7	8	9	9	0	1	2	2	3	6	6	6	7	8	0	0	0	0	1	
	5	3	9	0	1	8	9	3	6	6	0	1	2	8	7	4	7	7	7	1	1	2	8	9	4	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	3	2	0	1	4	1	0	5	0	1	1	1	4	0	4	4	1	5	1	2	4	3	4	2	5	
	6	9	2	3	4	5	6	4	1	1	4	2	0	7	8	9	0	3	8	2	5	8	2	7	0	
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Carcinoma, metastatic, thyroid gland																										
Carcinoma, metastatic, adrenal cortex																										
Osteosarcoma, metastatic, bone																						X				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	7	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Total
	3	2	2	2	2	2	3	4	5	5	0	0	0	2	2	3	3	4	5	1	1	1	3	3	4	Tissues/
	3	0	1	3	5	6	9	3	1	6	4	5	8	4	8	5	7	1	5	6	7	9	0	4	6	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland	X																									1
Carcinoma, metastatic, adrenal cortex									X																	1
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Respiratory System Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Ozone: 0.5 ppm

Number of Days on Study	1	3	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7
	2	7	3	5	5	9	1	3	5	5	5	6	6	9	9	0	0	0	2	3	3	3	3	3	3
	0	8	6	3	5	1	3	8	3	7	9	0	5	7	8	2	9	9	3	0	3	3	3	3	3
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	0	3	3	3	4	3	0	5	3	0	1	1	5	0	2	3	1	4	5	0	0	1	1	1	1
	8	7	8	3	3	9	6	1	0	9	9	8	4	5	2	1	0	4	2	4	2	2	3	4	7
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						
Histiocytic sarcoma															X										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glands, adenoma																		X							
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	Total
	2	2	2	2	4	4	0	1	1	2	2	2	3	3	4	4	5	0	1	3	3	4	5	5	5	Tissues/
	5	6	7	9	2	6	7	1	6	0	1	4	4	5	7	9	0	1	5	2	6	5	3	5	6	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																								X		2
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glands, adenoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Respiratory System Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Ozone: 1.0 ppm

Number of Days on Study	0	1	2	4	4	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	
	5	8	7	3	6	1	2	7	8	1	2	3	4	4	5	5	6	6	8	8	8	1	1	3	3	
	0	3	5	4	3	2	6	1	3	6	7	6	1	8	1	4	5	8	1	3	5	5	9	3	3	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	4	1	2	3	1	3	3	1	0	2	2	3	4	0	2	3	3	2	5	4	1	0	5	0	1	
	6	0	2	7	3	6	1	1	9	9	6	4	2	2	5	9	0	7	4	4	6	8	2	3	9	
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Carcinoma, metastatic, mammary gland																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	3	3	3	3	3	3	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	Total
	2	2	4	5	5	5	0	3	3	4	4	5	5	0	0	0	0	1	1	2	2	2	3	4	4	Tissues/
	0	1	3	1	5	6	6	3	8	1	7	0	3	1	4	5	7	2	7	3	4	8	2	0	8	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, mammary gland																					X					1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Ozone

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	6/50 (12%)	5/50 (10%)	6/50 (12%)	4/50 (8%)
Adjusted rate[b]	19.7%	16.8%	17.3%	14.8%
Terminal rate[c]	4/28 (14%)	2/24 (8%)	3/30 (10%)	4/27 (15%)
First incidence (days)	688	568	665	733 (T)
Life table test[d]	P=0.338N	P=0.580N	P=0.568N	P=0.402N
Logistic regression test[d]	P=0.351N	P=0.533N	P=0.574N	P=0.408N
Cochran-Armitage test[d]	P=0.349N			
Fisher exact test[d]		P=0.500N	P=0.620N	P=0.370N
Adrenal Medulla: Benign or Complex Pheochromocytoma				
Overall rate	6/50 (12%)	6/50 (12%)	6/50 (12%)	4/50 (8%)
Adjusted rate	19.7%	18.6%	17.3%	14.8%
Terminal rate	4/28 (14%)	2/24 (8%)	3/30 (10%)	4/27 (15%)
First incidence (days)	688	470	665	733 (T)
Life table test	P=0.285N	P=0.541	P=0.568N	P=0.402N
Logistic regression test	P=0.300N	P=0.609	P=0.574N	P=0.408N
Cochran-Armitage test	P=0.293N			
Fisher exact test		P=0.620N	P=0.620N	P=0.370N
Clitoral Gland: Adenoma				
Overall rate	5/43 (12%)	3/50 (6%)	8/47 (17%)	8/47 (17%)
Adjusted rate	21.6%	12.5%	24.9%	30.0%
Terminal rate	4/21 (19%)	3/24 (13%)	5/27 (19%)	6/24 (25%)
First incidence (days)	709	733 (T)	659	685
Life table test	P=0.112	P=0.301N	P=0.407	P=0.329
Logistic regression test	P=0.092	P=0.277N	P=0.418	P=0.315
Cochran-Armitage test	P=0.098			
Fisher exact test		P=0.276N	P=0.336	P=0.336
Clitoral Gland: Carcinoma				
Overall rate	0/43 (0%)	3/50 (6%)	3/47 (6%)	1/47 (2%)
Adjusted rate	0.0%	12.5%	11.1%	4.2%
Terminal rate	0/21 (0%)	3/24 (13%)	3/27 (11%)	1/24 (4%)
First incidence (days)	–[e]	733 (T)	733 (T)	733 (T)
Life table test	P=0.567N	P=0.143	P=0.167	P=0.527
Logistic regression test	P=0.567N	P=0.143	P=0.167	P=0.527
Cochran-Armitage test	P=0.597N			
Fisher exact test		P=0.151	P=0.138	P=0.522
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	5/43 (12%)	5/50 (10%)	11/47 (23%)	9/47 (19%)
Adjusted rate	21.6%	20.8%	35.2%	33.9%
Terminal rate	4/21 (19%)	5/24 (21%)	8/27 (30%)	7/24 (29%)
First incidence (days)	709	733 (T)	659	685
Life table test	P=0.107	P=0.567N	P=0.172	P=0.240
Logistic regression test	P=0.085	P=0.545N	P=0.173	P=0.222
Cochran-Armitage test	P=0.093			
Fisher exact test		P=0.530N	P=0.118	P=0.246

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Mammary Gland: Carcinoma				
Overall rate	4/50 (8%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	14.3%	4.2%	8.1%	3.7%
Terminal rate	4/28 (14%)	1/24 (4%)	1/30 (3%)	1/27 (4%)
First incidence (days)	733 (T)	733 (T)	536	733 (T)
Life table test	P=0.237N	P=0.225N	P=0.461N	P=0.187N
Logistic regression test	P=0.251N	P=0.223N	P=0.484N	P=0.187N
Cochran-Armitage test	P=0.249N			
Fisher exact test		P=0.181N	P=0.500N	P=0.181N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	2/50 (4%)	4/50 (8%)	1/50 (2%)
Adjusted rate	17.9%	8.3%	10.4%	3.7%
Terminal rate	5/28 (18%)	2/24 (8%)	1/30 (3%)	1/27 (4%)
First incidence (days)	733 (T)	733 (T)	536	733 (T)
Life table test	P=0.139N	P=0.278N	P=0.459N	P=0.108N
Logistic regression test	P=0.148N	P=0.278N	P=0.483N	P=0.108N
Cochran-Armitage test	P=0.148N			
Fisher exact test		P=0.218N	P=0.500N	P=0.102N
Mammary Gland: Fibroadenoma				
Overall rate	20/50 (40%)	17/50 (34%)	23/50 (46%)	12/50 (24%)
Adjusted rate	54.6%	57.1%	59.6%	40.4%
Terminal rate	12/28 (43%)	12/24 (50%)	15/30 (50%)	10/27 (37%)
First incidence (days)	571	596	536	512
Life table test	P=0.092N	P=0.533N	P=0.461	P=0.099N
Logistic regression test	P=0.099N	P=0.393N	P=0.414	P=0.078N
Cochran-Armitage test	P=0.102N			
Fisher exact test		P=0.339N	P=0.343	P=0.066N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	21/50 (42%)	18/50 (36%)	24/50 (48%)	12/50 (24%)
Adjusted rate	57.5%	60.6%	60.6%	40.4%
Terminal rate	13/28 (46%)	13/24 (54%)	15/30 (50%)	10/27 (37%)
First incidence (days)	571	596	536	512
Life table test	P=0.063N	P=0.545N	P=0.465	P=0.069N
Logistic regression test	P=0.065N	P=0.400N	P=0.416	P=0.052N
Cochran-Armitage test	P=0.069N			
Fisher exact test		P=0.341N	P=0.344	P=0.044N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	23/50 (46%)	19/50 (38%)	25/50 (50%)	13/50 (26%)
Adjusted rate	63.1%	64.2%	63.3%	43.9%
Terminal rate	15/28 (54%)	14/24 (58%)	16/30 (53%)	11/27 (41%)
First incidence (days)	571	596	536	512
Life table test	P=0.046N	P=0.485N	P=0.544	P=0.048N
Logistic regression test	P=0.047N	P=0.328N	P=0.513	P=0.035N
Cochran-Armitage test	P=0.053N			
Fisher exact test		P=0.272N	P=0.421	P=0.030N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	34/50 (68%)	36/49 (73%)	38/50 (76%)	33/49 (67%)
Adjusted rate	86.7%	94.5%	88.3%	88.8%
Terminal rate	23/28 (82%)	22/24 (92%)	25/30 (83%)	22/26 (85%)
First incidence (days)	502	469	536	571
Life table test	P=0.423N	P=0.168	P=0.463	P=0.485
Logistic regression test	P=0.517N	P=0.277	P=0.359	P=0.504
Cochran-Armitage test	P=0.450N			
Fisher exact test		P=0.353	P=0.252	P=0.558N
Thyroid Gland (C-cell): Adenoma				
Overall rate	5/50 (10%)	5/50 (10%)	5/50 (10%)	2/50 (4%)
Adjusted rate	15.8%	19.4%	16.7%	5.4%
Terminal rate	3/28 (11%)	4/24 (17%)	5/30 (17%)	0/27 (0%)
First incidence (days)	667	681	733 (T)	648
Life table test	P=0.148N	P=0.534	P=0.591N	P=0.246N
Logistic regression test	P=0.157N	P=0.586	P=0.583N	P=0.230N
Cochran-Armitage test	P=0.159N			
Fisher exact test		P=0.630N	P=0.630N	P=0.218N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	5/50 (10%)	6/50 (12%)	7/50 (14%)	2/50 (4%)
Adjusted rate	15.8%	22.6%	23.3%	5.4%
Terminal rate	3/28 (11%)	4/24 (17%)	7/30 (23%)	0/27 (0%)
First incidence (days)	667	681	733 (T)	648
Life table test	P=0.150N	P=0.401	P=0.423	P=0.246N
Logistic regression test	P=0.159N	P=0.447	P=0.434	P=0.230N
Cochran-Armitage test	P=0.162N			
Fisher exact test		P=0.500	P=0.380	P=0.218N
Uterus: Stromal Polyp				
Overall rate	10/50 (20%)	12/50 (24%)	8/50 (16%)	7/50 (14%)
Adjusted rate	32.4%	36.9%	23.2%	22.5%
Terminal rate	8/28 (29%)	6/24 (25%)	5/30 (17%)	4/27 (15%)
First incidence (days)	665	611	613	654
Life table test	P=0.133N	P=0.291	P=0.337N	P=0.340N
Logistic regression test	P=0.144N	P=0.365	P=0.337N	P=0.330N
Cochran-Armitage test	P=0.142N			
Fisher exact test		P=0.405	P=0.398N	P=0.298N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	11/50 (22%)	12/50 (24%)	8/50 (16%)	7/50 (14%)
Adjusted rate	33.9%	36.9%	23.2%	22.5%
Terminal rate	8/28 (29%)	6/24 (25%)	5/30 (17%)	4/27 (15%)
First incidence (days)	527	611	613	654
Life table test	P=0.102N	P=0.378	P=0.254N	P=0.260N
Logistic regression test	P=0.110N	P=0.472	P=0.268N	P=0.242N
Cochran-Armitage test	P=0.106N			
Fisher exact test		P=0.500	P=0.306N	P=0.218N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
All Organs: Mononuclear Cell Leukemia				
Overall rate	17/50 (34%)	18/50 (36%)	16/50 (32%)	17/50 (34%)
Adjusted rate	42.6	45.0%	40.8%	43.6%
Terminal rate	7/28 (25%)	5/24 (21%)	8/30 (27%)	7/27 (26%)
First incidence (days)	443	541	378	434
Life table test	P=0.471N	P=0.399	P=0.424N	P=0.509
Logistic regression test	P=0.491	P=0.561N	P=0.506N	P=0.575N
Cochran-Armitage test	P=0.480N			
Fisher exact test		P=0.500	P=0.500N	P=0.583N
All Organs: Benign Neoplasms				
Overall rate	41/50 (82%)	43/50 (86%)	45/50 (90%)	41/50 (82%)
Adjusted rate	97.6%	100.0%	97.8%	95.3%
Terminal rate	27/28 (96%)	24/24 (100%)	29/30 (97%)	25/27 (93%)
First incidence (days)	502	469	378	512
Life table test	P=0.427N	P=0.150	P=0.497	P=0.453
Logistic regression test	P=0.467	P=0.327	P=0.304	P=0.467
Cochran-Armitage test	P=0.512N			
Fisher exact test		P=0.393	P=0.194	P=0.602N
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	28/50 (56%)	26/50 (52%)	23/50 (46%)
Adjusted rate	60.1%	68.5%	61.1%	55.9%
Terminal rate	12/28 (43%)	12/24 (50%)	14/30 (47%)	10/27 (37%)
First incidence (days)	443	470	378	275
Life table test	P=0.256N	P=0.295	P=0.461N	P=0.449N
Logistic regression test	P=0.316N	P=0.431	P=0.564	P=0.403N
Cochran-Armitage test	P=0.223N			
Fisher exact test		P=0.421	P=0.579N	P=0.345N
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	48/50 (96%)	48/50 (96%)	46/50 (92%)
Adjusted rate	98.0%	100.0%	100.0%	97.9%
Terminal rate	27/28 (96%)	24/24 (100%)	30/30 (100%)	26/27 (96%)
First incidence (days)	443	469	378	275
Life table test	P=0.335N	P=0.268	P=0.363N	P=0.559
Logistic regression test	P=0.531N	P=0.660	P=0.675	P=0.588N
Cochran-Armitage test	P=0.217N			
Fisher exact test		P=0.691N	P=0.691N	P=0.339N

(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, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.

[e] Not applicable; no neoplasms in animal group

TABLE B4
Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Northwest			
o-Chlorobenzalmalononitrile	2/49	0/49	2/49
α-Chloroacetophenone	1/49	0/49	1/49
Epinephrine hydrochloride	0/50	0/50	0/50
Ethyl chloride	0/50	0/50	0/50
Hexachlorocyclopentadiene	1/50	0/50	1/50
Overall Historical Incidence			
Total	4/398 (1.0%)	0/398	4/398 (1.0%)
Standard deviation	1.5%		1.5%
Range	0%-4%		0%-4%

[a] Data as of 31 March 1993

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Ozone[a]

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	19	22	17	16
Natural deaths	3	4	3	7
Survivors				
Terminal sacrifice	28	24	30	27
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(50)	(50)	(50)	(49)
Cyst				1 (2%)
Parasite metazoan	2 (4%)	2 (4%)	3 (6%)	5 (10%)
Intestine large, rectum	(43)	(48)	(45)	(48)
Parasite metazoan	4 (9%)	4 (8%)	3 (7%)	1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Parasite metazoan	4 (8%)	2 (4%)	8 (16%)	9 (18%)
Intestine small, duodenum	(50)	(50)	(50)	(49)
Parasite metazoan			1 (2%)	
Intestine small, ileum	(50)	(49)	(48)	(47)
Parasite metazoan	1 (2%)	1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	2 (4%)		4 (8%)
Basophilic focus	38 (76%)	34 (68%)	35 (70%)	38 (76%)
Clear cell focus	6 (12%)	5 (10%)	5 (10%)	4 (8%)
Degeneration, fatty	13 (26%)	6 (12%)	8 (16%)	4 (8%)
Eosinophilic focus	3 (6%)	1 (2%)		2 (4%)
Hepatodiaphragmatic nodule	7 (14%)	6 (12%)	6 (12%)	15 (30%)
Mixed cell focus	5 (10%)	13 (26%)	6 (12%)	7 (14%)
Necrosis	1 (2%)		1 (2%)	
Regeneration				1 (2%)
Bile duct, hyperplasia	10 (20%)	11 (22%)	8 (16%)	11 (22%)
Centrilobular, necrosis	2 (4%)	4 (8%)	3 (6%)	1 (2%)
Serosa, fibrosis			1 (2%)	
Mesentery	(4)	(5)	(11)	(4)
Hemorrhage	1 (25%)			
Artery, inflammation, chronic active	1 (25%)		1 (9%)	1 (25%)
Artery, mineralization	1 (25%)			
Fat, necrosis	2 (50%)	5 (100%)	9 (82%)	3 (75%)
Oral mucosa	(1)	(1)		
Pharyngeal, hyperplasia	1 (100%)			
Pancreas	(50)	(50)	(50)	(49)
Atrophy	22 (44%)	14 (28%)	18 (36%)	20 (41%)
Basophilic focus	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Metaplasia, hepatocyte		1 (2%)		
Artery, inflammation				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Duct, metaplasia, squamous				1 (2%)

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

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum	1 (2%)			1 (2%)
Hyperplasia, squamous			1 (2%)	1 (2%)
Mineralization			2 (4%)	
Necrosis	2 (4%)	4 (8%)	4 (8%)	3 (6%)
Stomach, glandular	(50)	(50)	(50)	(50)
Inflammation, acute				1 (2%)
Mineralization	5 (10%)	4 (8%)	5 (10%)	7 (14%)
Necrosis		4 (8%)	2 (4%)	2 (4%)
Tongue	(1)	(1)		(1)
Hyperplasia				1 (100%)
Tooth		(1)	(1)	(1)
Developmental malformation		1 (100%)	1 (100%)	1 (100%)
Inflammation, chronic active		1 (100%)		
Cardiovascular System				
Blood vessel	(1)			
Aorta, mineralization	1 (100%)			
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	37 (74%)	32 (64%)	34 (68%)	30 (60%)
Artery, mineralization	1 (2%)			
Atrium, thrombosis	1 (2%)	1 (2%)	2 (4%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hyperplasia	21 (42%)	22 (44%)	22 (44%)	23 (46%)
Hypertrophy	12 (24%)	13 (26%)	9 (18%)	7 (14%)
Mineralization				1 (2%)
Necrosis	1 (2%)			1 (2%)
Vacuolization cytoplasmic			2 (4%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	9 (18%)	10 (20%)	11 (22%)	5 (10%)
Parathyroid gland	(49)	(48)	(46)	(49)
Hyperplasia	3 (6%)	1 (2%)	3 (7%)	1 (2%)
Pituitary gland	(50)	(49)	(50)	(49)
Cyst	5 (10%)		1 (2%)	
Pars distalis, hyperplasia	13 (26%)	8 (16%)	7 (14%)	10 (20%)
Pars intermedia, hyperplasia		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	43 (86%)	38 (76%)	34 (68%)	31 (62%)
Follicular cell, hyperplasia	2 (4%)		4 (8%)	1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(43)	(50)	(47)	(47)
Cyst				1 (2%)
Hyperplasia		1 (2%)	2 (4%)	
Inflammation, chronic active		3 (6%)	2 (4%)	4 (9%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Genital System (continued)				
Ovary	(50)	(50)	(49)	(49)
Angiectasis	1 (2%)			
Cyst	1 (2%)	2 (4%)	11 (22%)	1 (2%)
Hemorrhage				1 (2%)
Inflammation, granulomatous	2 (4%)		1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia, reticulum cell	1 (2%)	2 (4%)		4 (8%)
Lymph node	(5)	(9)	(3)	(3)
Renal, hemorrhage		1 (11%)		1 (33%)
Renal, inflammation, granulomatous		1 (11%)	1 (33%)	
Lymph node, mandibular	(48)	(47)	(46)	(46)
Infiltration cellular, plasma cell		1 (2%)		1 (2%)
Lymph node, mesenteric	(49)	(50)	(49)	(50)
Hemorrhage		1 (2%)	1 (2%)	
Lymph node, mediastinal	(39)	(46)	(41)	(47)
Hemorrhage	1 (3%)		1 (2%)	
Spleen	(50)	(50)	(50)	(49)
Fibrosis	2 (4%)	4 (8%)	2 (4%)	
Hematopoietic cell proliferation	2 (4%)		2 (4%)	2 (4%)
Hemorrhage	1 (2%)			1 (2%)
Necrosis	2 (4%)	1 (2%)		
Capsule, fibrosis			1 (2%)	
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Galactocele		2 (4%)		
Hyperplasia, atypical	4 (8%)		1 (2%)	
Inflammation, suppurative		1 (2%)		
Skin	(50)	(50)	(50)	(50)
Hyperkeratosis	1 (2%)	1 (2%)		
Inflammation, chronic active	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Prepuce, inflammation, acute	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy	1 (2%)			1 (2%)
Hyperostosis	3 (6%)	2 (4%)	6 (12%)	6 (12%)
Skeletal muscle	(1)			(3)
Cyst				1 (33%)
Hemorrhage	1 (100%)			1 (33%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Necrosis		1 (2%)		
Meninges, hyperplasia	1 (2%)			1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Inflammation, acute	1 (2%)	1 (2%)		1 (2%)
Mineralization	1 (2%)			
Epiglottis, metaplasia, squamous	4 (8%)	5 (10%)	9 (18%)	43 (86%)
Lung	(50)	(50)	(50)	(50)
Inflammation, chronic active	5 (10%)	3 (6%)		
Inflammation, suppurative		1 (2%)		
Mineralization	2 (4%)			1 (2%)
Alveolar epithelium, hyperplasia	4 (8%)	5 (10%)	5 (10%)	6 (12%)
Alveolar epithelium, metaplasia		6 (12%)	48 (96%)	48 (96%)
Alveolus, infiltration cellular, histiocyte			31 (62%)	43 (86%)
Artery, mediastinum, mineralization	1 (2%)			
Interstitium, fibrosis			42 (84%)	47 (94%)
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	3 (6%)	6 (12%)	2 (4%)	2 (4%)
Necrosis		1 (2%)		
Thrombosis	6 (12%)	3 (6%)	4 (8%)	3 (6%)
Goblet cell, lateral wall, hyperplasia	1 (2%)	2 (4%)	45 (90%)	50 (100%)
Lateral wall, hyperplasia	2 (4%)	8 (16%)	48 (96%)	50 (100%)
Lateral wall, metaplasia, squamous	2 (4%)	11 (22%)	21 (42%)	45 (90%)
Nasopharyngeal duct, inflammation, acute	1 (2%)			
Olfactory epithelium, degeneration, hyaline	50 (100%)	48 (96%)	50 (100%)	47 (94%)
Olfactory epithelium, metaplasia		3 (6%)		
Trachea	(50)	(50)	(50)	(50)
Inflammation, acute	1 (2%)			
Mineralization	1 (2%)			
Special Senses System				
Eye		(1)		(2)
Cataract				2 (100%)
Degeneration		1 (100%)		
Retina, atrophy				1 (50%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst			1 (2%)	1 (2%)
Hemorrhage				1 (2%)
Infarct	1 (2%)			
Mineralization	1 (2%)			
Nephropathy	49 (98%)	48 (96%)	48 (96%)	46 (92%)
Renal tubule, hyperplasia			1 (2%)	1 (2%)
Renal tubule, vacuolization cytoplasmic		1 (2%)		
Urinary bladder	(50)	(49)	(49)	(50)
Hemorrhage		1 (2%)		
Necrosis		1 (2%)		

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

TABLE C1 Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Ozone 126

TABLE C2 Individual Animal Respiratory System Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Ozone 129

TABLE C3 Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Ozone 133

TABLE C4 Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Male $B6C3F_1$ Mice 137

TABLE C5 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Ozone 138

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

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	16	10	19	20
Natural deaths	4	6	6	3
Survivors				
Died last week of study	1			
Terminal sacrifice	29	34	25	27
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(46)	(46)	(48)	(48)
Adenoma			1 (2%)	
Carcinoma, metastatic, uncertain primary site	1 (2%)			
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(49)	(50)	(50)
Intestine small, duodenum	(49)	(47)	(48)	(48)
Intestine small, jejunum	(49)	(48)	(49)	(49)
Carcinoma	1 (2%)			
Intestine small, ileum	(49)	(48)	(49)	(50)
Carcinoma			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma		3 (6%)	1 (2%)	
Hepatocellular carcinoma	10 (20%)	4 (8%)	11 (22%)	13 (26%)
Hepatocellular carcinoma, multiple	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Hepatocellular adenoma	9 (18%)	17 (34%)	13 (26%)	12 (24%)
Hepatocellular adenoma, multiple	14 (28%)	4 (8%)	6 (12%)	4 (8%)
Mesentery	(4)	(5)	(1)	(3)
Carcinoma, metastatic, uncertain primary site	1 (25%)			
Hemangiosarcoma		1 (20%)		
Pancreas	(49)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma		1 (2%)		
Squamous cell papilloma		2 (4%)		
Stomach, glandular	(50)	(49)	(50)	(50)
Tooth	(1)	(1)	(1)	(1)
Odontoma		1 (100%)	1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant		1 (2%)		
Pheochromocytoma benign				1 (2%)
Islets, pancreatic	(49)	(50)	(49)	(50)
Adenoma		2 (4%)		1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Endocrine System (continued)				
Pituitary gland	(47)	(49)	(49)	(48)
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(49)	(50)	(50)	(50)
Follicular cell, adenoma			1 (2%)	
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(49)	(50)	(47)	(49)
Seminal vesicle	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uncertain primary site	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma		2 (4%)		
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Mast cell tumor benign				1 (2%)
Lymph node	(4)	(6)	(3)	(10)
Lymph node, bronchial	(40)	(43)	(36)	(38)
Hepatocellular carcinoma, metastatic, liver			1 (3%)	
Lymph node, mandibular	(41)	(43)	(41)	(38)
Lymph node, mesenteric	(48)	(49)	(49)	(49)
Carcinoma, metastatic, uncertain primary site	1 (2%)			
Lymph node, mediastinal	(40)	(48)	(39)	(45)
Hepatocellular carcinoma, metastatic, liver			1 (3%)	
Spleen	(49)	(50)	(49)	(50)
Carcinoma, metastatic, uncertain primary site	1 (2%)			
Hemangiosarcoma		1 (2%)	1 (2%)	
Histiocytic sarcoma		1 (2%)		
Thymus	(39)	(42)	(37)	(41)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, hemangioma			1 (2%)	
Subcutaneous tissue, lipoma		1 (2%)		
Subcutaneous tissue, sarcoma	1 (2%)			1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteoma			1 (2%)	
Skeletal muscle			(1)	(1)
Hemangioma				1 (100%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	6 (12%)	9 (18%)	9 (18%)	10 (20%)
Alveolar/bronchiolar adenoma, multiple			3 (6%)	1 (2%)
Alveolar/bronchiolar carcinoma	6 (12%)	2 (4%)	4 (8%)	8 (16%)
Alveolar/bronchiolar carcinoma, multiple	2 (4%)	2 (4%)	4 (8%)	2 (4%)
Carcinoma, metastatic, harderian gland		1 (2%)		
Hepatocellular carcinoma, metastatic, liver	5 (10%)	2 (4%)	4 (8%)	2 (4%)
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Harderian gland	(1)	(8)	(6)	(4)
Adenoma	1 (100%)	4 (50%)	3 (50%)	4 (100%)
Carcinoma		3 (38%)	3 (50%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma	1 (2%)	1 (2%)		
Renal tubule, carcinoma	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(49)
Carcinoma, metastatic, urinary bladder	1 (2%)			
Transitional epithelium, papilloma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Lymphoma malignant	4 (8%)	7 (14%)	4 (8%)	7 (14%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	39	43	42	40
Total primary neoplasms	60	71	70	68
Total animals with benign neoplasms	27	33	30	29
Total benign neoplasms	33	43	39	35
Total animals with malignant neoplasms	24	25	24	27
Total malignant neoplasms	27	28	31	33
Total animals with metastatic neoplasms	6	3	4	2
Total metastatic neoplasms	11	3	6	2
Total animals with malignant neoplasms uncertain primary site	1			
Total uncertain neoplasms	1			

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

TABLE C2
Individual Animal Respiratory System Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Ozone: 0 ppm

Number of Days on Study	205	362	446	453	484	525	596	602	608	609	611	612	644	644	653	667	681	695	701	729	733	733	733	733	733
Carcass ID Number	0048	0011	0008	0027	0010	0018	0031	0006	0009	0032	0043	0045	0044	0047	0015	0007	0011	0012	0021	0022	0040	0017	0022	0023	0024
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X													X	
Alveolar/bronchiolar carcinoma																						X			
Alveolar/bronchiolar carcinoma, multiple															X										
Hepatocellular carcinoma, metastatic, liver		X			X	X														X					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	
Carcass ID Number	0026	0028	0030	0033	0035	0038	0040	0041	0046	0049	0053	0054	0055	0053	0054	0056	0059	0060	0065	0069	0034	0036	0037	0039	0050	Total Tissues/ Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X												X	X									X	6
Alveolar/bronchiolar carcinoma					X	X		X			X											X				6
Alveolar/bronchiolar carcinoma, multiple		X																								2
Hepatocellular carcinoma, metastatic, liver																	X									5
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

+: Tissue examined microscopically

X: Lesion present

TABLE C2
Individual Animal Respiratory System Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Ozone: 0.12 ppm

Number of Days on Study	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7
	4	5	2	4	4	5	6	9	0	1	3	6	8	9	9	9	3	3	3	3	3	3	3	3	3
	0	9	7	0	5	1	7	6	0	2	7	7	1	5	5	9	3	3	3	3	3	3	3	3	3
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	2	2	0	2	1	4	1	0	2	0	3	4	0	1	1	1	0	0	0	1	1	1	1	2	2
	3	9	6	6	1	6	0	3	4	4	6	4	2	5	6	4	1	5	9	3	7	8	9	0	1
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma	X																					X		X	X
Alveolar/bronchiolar carcinoma													X												
Alveolar/bronchiolar carcinoma, multiple										X				X											
Carcinoma, metastatic, harderian gland																									
Hepatocellular carcinoma, metastatic, liver			X				X																		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	3	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Total
	2	2	3	3	3	3	4	4	4	4	4	4	4	0	0	1	2	2	3	3	3	3	3	4	5	Tissues/
	2	7	1	4	5	9	0	1	2	3	5	7	8	7	8	2	5	8	0	2	3	7	8	9	0	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X		X	X							X							X			9
Alveolar/bronchiolar carcinoma			X																							2
Alveolar/bronchiolar carcinoma, multiple																										2
Carcinoma, metastatic, harderian gland																X										1
Hepatocellular carcinoma, metastatic, liver																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Respiratory System Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Ozone: 0.5 ppm

Number of Days on Study	1	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	7	7	
	4	6	6	6	8	8	8	2	2	4	5	8	8	8	9	9	2	3	4	5	5	6	8	0	0	
	5	0	1	4	4	4	9	2	7	4	9	3	3	3	6	6	1	0	4	3	9	5	1	1	9	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
	1	3	4	0	0	1	0	2	3	1	0	1	2	4	3	5	2	4	1	1	4	3	2	4	3	
	0	1	8	4	9	8	7	5	4	2	6	7	2	1	9	0	8	7	4	1	3	2	9	6	3	
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma				X								X								X						
Alveolar/bronchiolar adenoma, multiple																										
Alveolar/bronchiolar carcinoma																								X		
Alveolar/bronchiolar carcinoma, multiple																										
Hepatocellular carcinoma, metastatic, liver	X								X															X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	Total
	0	1	2	2	2	3	4	4	0	0	0	0	1	1	1	2	2	2	3	3	3	3	4	4	4	Tissues/
	5	3	1	4	6	6	2	4	1	2	3	8	5	6	9	0	3	7	0	5	7	8	0	5	9	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma					X	X			X							X				X					X	9
Alveolar/bronchiolar adenoma, multiple	X							X																X		3
Alveolar/bronchiolar carcinoma																			X				X		X	4
Alveolar/bronchiolar carcinoma, multiple	X										X		X					X								4
Hepatocellular carcinoma, metastatic, liver											X															4
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Respiratory System Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Ozone: 1.0 ppm

Number of Days on Study	0	3	3	4	4	4	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	7	7	7
	8	5	8	4	6	8	0	1	2	5	5	6	9	9	0	1	1	1	3	5	6	8	0	3	3
	0	0	0	6	2	4	6	4	5	1	7	7	2	5	2	2	2	6	0	8	5	1	9	3	3
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	2	0	4	1	0	2	3	1	1	1	3	0	4	4	1	0	2	4	3	5	4	4	1	0	0
	0	1	1	8	4	4	6	2	7	1	3	9	4	2	3	3	3	7	9	0	0	5	6	5	8
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X											X							X	
Alveolar/bronchiolar adenoma, multiple							X																		
Alveolar/bronchiolar carcinoma																			X						
Alveolar/bronchiolar carcinoma, multiple																									
Hepatocellular carcinoma, metastatic, liver																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	Total
	1	1	1	1	2	2	2	3	3	3	3	4	4	4	0	0	0	2	2	2	2	3	3	3	4	Tissues/
	0	4	5	9	1	5	8	1	2	7	8	3	6	8	2	6	7	2	6	7	9	0	4	5	9	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X			X							X	X						X				X	X	10
Alveolar/bronchiolar adenoma, multiple																										1
Alveolar/bronchiolar carcinoma	X			X			X					X							X	X			X			8
Alveolar/bronchiolar carcinoma, multiple		X	X																							2
Hepatocellular carcinoma, metastatic, liver																					X		X			2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Ozone

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Harderian Gland: Adenoma				
Overall rate[a]	1/50 (2%)	4/50 (8%)	3/50 (6%)	4/50 (8%)
Adjusted rate[b]	2.0%	11.8%	9.7%	13.1%
Terminal rate[c]	0/30 (0%)	4/34 (12%)	1/25 (4%)	3/27 (11%)
First incidence (days)	362	733 (T)	621	506
Life table test[d]	P=0.183	P=0.213	P=0.268	P=0.162
Logistic regression test[d]	P=0.246	P=0.164	P=0.335	P=0.199
Cochran-Armitage test[d]	P=0.253			
Fisher exact test[d]		P=0.181	P=0.309	P=0.181
Harderian Gland: Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	8.8%	10.5%	0.0%
Terminal rate	0/30 (0%)	3/34 (9%)	1/25 (4%)	0/27 (0%)
First incidence (days)	–[e]	733 (T)	659	–
Life table test	P=0.444N	P=0.143	P=0.095	–
Logistic regression test	P=0.425N	P=0.143	P=0.107	–
Cochran-Armitage test	P=0.367N			
Fisher exact test		P=0.121	P=0.121	–
Harderian Gland: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	7/50 (14%)	6/50 (12%)	4/50 (8%)
Adjusted rate	2.0%	20.6%	19.4%	13.1%
Terminal rate	0/30 (0%)	7/34 (21%)	2/25 (8%)	3/27 (11%)
First incidence (days)	362	733 (T)	621	506
Life table test	P=0.306	P=0.046	P=0.044	P=0.162
Logistic regression test	P=0.384	P=0.034	P=0.064	P=0.199
Cochran-Armitage test	P=0.425			
Fisher exact test		P=0.030	P=0.056	P=0.181
Liver: Hemangiosarcoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	7.2%	3.3%	0.0%
Terminal rate	0/30 (0%)	1/34 (3%)	0/25 (0%)	0/27 (0%)
First incidence (days)	–	540	659	–
Life table test	P=0.303N	P=0.134	P=0.469	–
Logistic regression test	P=0.231N	P=0.091	P=0.500	–
Cochran-Armitage test	P=0.254N			
Fisher exact test		P=0.121	P=0.500	–
Liver: Hepatocellular Adenoma				
Overall rate	23/50 (46%)	21/50 (42%)	19/50 (38%)	16/50 (32%)
Adjusted rate	65.0%	53.6%	59.5%	43.3%
Terminal rate	18/30 (60%)	16/34 (47%)	13/25 (52%)	8/27 (30%)
First incidence (days)	446	545	484	462
Life table test	P=0.293N	P=0.250N	P=0.541N	P=0.229N
Logistic regression test	P=0.159N	P=0.333N	P=0.401N	P=0.148N
Cochran-Armitage test	P=0.084N			
Fisher exact test		P=0.420N	P=0.272N	P=0.109N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Liver: Hepatocellular Carcinoma				
Overall rate	12/50 (24%)	6/50 (12%)	13/50 (26%)	15/50 (30%)
Adjusted rate	29.2%	13.1%	31.8%	38.5%
Terminal rate	4/30 (13%)	1/34 (3%)	2/25 (8%)	5/27 (19%)
First incidence (days)	362	440	460	350
Life table test	P=0.047	P=0.091N	P=0.391	P=0.258
Logistic regression test	P=0.188	P=0.100N	P=0.469N	P=0.392
Cochran-Armitage test	P=0.075			
Fisher exact test		P=0.096N	P=0.500	P=0.326
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	30/50 (60%)	27/50 (54%)	29/50 (58%)	29/50 (58%)
Adjusted rate	70.7%	60.7%	70.8%	66.9%
Terminal rate	18/30 (60%)	17/34 (50%)	14/25 (56%)	13/27 (48%)
First incidence (days)	362	440	460	350
Life table test	P=0.206	P=0.218N	P=0.362	P=0.434
Logistic regression test	P=0.517	P=0.356N	P=0.506N	P=0.512N
Cochran-Armitage test	P=0.512			
Fisher exact test		P=0.343N	P=0.500N	P=0.500N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/50 (12%)	9/50 (18%)	12/50 (24%)	11/50 (22%)
Adjusted rate	18.8%	25.1%	40.9%	34.7%
Terminal rate	5/30 (17%)	8/34 (24%)	9/25 (36%)	8/27 (30%)
First incidence (days)	611	440	464	484
Life table test	P=0.053	P=0.372	P=0.045	P=0.100
Logistic regression test	P=0.079	P=0.318	P=0.061	P=0.110
Cochran-Armitage test	P=0.130			
Fisher exact test		P=0.288	P=0.096	P=0.143
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	8/50 (16%)	4/50 (8%)	8/50 (16%)	10/50 (20%)
Adjusted rate	25.5%	10.3%	30.7%	35.4%
Terminal rate	7/30 (23%)	1/34 (3%)	7/25 (28%)	9/27 (33%)
First incidence (days)	653	612	701	630
Life table test	P=0.063	P=0.135N	P=0.451	P=0.294
Logistic regression test	P=0.062	P=0.154N	P=0.449	P=0.270
Cochran-Armitage test	P=0.145			
Fisher exact test		P=0.178N	P=0.607N	P=0.398
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	14/50 (28%)	13/50 (26%)	18/50 (36%)	19/50 (38%)
Adjusted rate	43.1%	33.4%	60.9%	60.0%
Terminal rate	12/30 (40%)	9/34 (26%)	14/25 (56%)	15/27 (56%)
First incidence (days)	611	440	464	484
Life table test	P=0.020	P=0.368N	P=0.099	P=0.103
Logistic regression test	P=0.030	P=0.445N	P=0.124	P=0.103
Cochran-Armitage test	P=0.094			
Fisher exact test		P=0.500N	P=0.260	P=0.198

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	8.3%	0.0%	0.0%
Terminal rate	0/30 (0%)	2/34 (6%)	0/25 (0%)	0/27 (0%)
First incidence (days)	–	667	–	–
Life table test	P=0.224N	P=0.145	–	–
Logistic regression test	P=0.210N	P=0.129	–	–
Cochran-Armitage test	P=0.183N			
Fisher exact test		P=0.121	–	–
All Organs: Hemangiosarcoma				
Overall rate	0/50 (0%)	5/50 (10%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	12.6%	3.3%	0.0%
Terminal rate	0/30 (0%)	2/34 (6%)	0/25 (0%)	0/27 (0%)
First incidence (days)	–	540	659	–
Life table test	P=0.168N	P=0.044	P=0.469	–
Logistic regression test	P=0.122N	P=0.028	P=0.500	–
Cochran-Armitage test	P=0.125N			
Fisher exact test		P=0.028	P=0.500	–
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	0/50 (0%)	5/50 (10%)	2/50 (4%)	1/50 (2%)
Adjusted rate	0.0%	12.6%	7.2%	3.7%
Terminal rate	0/30 (0%)	2/34 (6%)	1/25 (4%)	1/27 (4%)
First incidence (days)	–	540	659	733 (T)
Life table test	P=0.442N	P=0.044	P=0.204	P=0.479
Logistic regression test	P=0.378N	P=0.028	P=0.218	P=0.479
Cochran-Armitage test	P=0.359N			
Fisher exact test		P=0.028	P=0.247	P=0.500
All Organs: Malignant Lymphoma (Histiocytic or Lymphocytic)				
Overall rate	4/50 (8%)	7/50 (14%)	4/50 (8%)	7/50 (14%)
Adjusted rate	10.8%	18.2%	12.5%	19.8%
Terminal rate	1/30 (3%)	4/34 (12%)	2/25 (8%)	3/27 (11%)
First incidence (days)	596	600	544	484
Life table test	P=0.238	P=0.315	P=0.554	P=0.213
Logistic regression test	P=0.372	P=0.262	P=0.640N	P=0.275
Cochran-Armitage test	P=0.347			
Fisher exact test		P=0.262	P=0.643N	P=0.262
All Organs: Malignant Lymphoma or Histiocytic Sarcoma				
Overall rate	4/50 (8%)	8/50 (16%)	4/50 (8%)	7/50 (14%)
Adjusted rate	10.8%	21.0%	12.5%	19.8%
Terminal rate	1/30 (3%)	5/34 (15%)	2/25 (8%)	3/27 (11%)
First incidence (days)	596	600	544	484
Life table test	P=0.289	P=0.229	P=0.554	P=0.213
Logistic regression test	P=0.411	P=0.183	P=0.640N	P=0.275
Cochran-Armitage test	P=0.411			
Fisher exact test		P=0.178	P=0.643N	P=0.262

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
All Organs: Benign Neoplasms				
Overall rate	27/50 (54%)	33/50 (66%)	30/50 (60%)	29/50 (58%)
Adjusted rate	72.1%	80.3%	84.9%	75.2%
Terminal rate	20/30 (67%)	26/34 (76%)	20/25 (80%)	18/27 (67%)
First incidence (days)	362	440	464	462
Life table test	P=0.174	P=0.369	P=0.105	P=0.239
Logistic regression test	P=0.347	P=0.206	P=0.224	P=0.321
Cochran-Armitage test	P=0.502N			
Fisher exact test		P=0.154	P=0.343	P=0.420
All Organs: Malignant Neoplasms				
Overall rate	25/50 (50%)	25/50 (50%)	25/50 (50%)	27/50 (54%)
Adjusted rate	58.5%	53.7%	60.3%	66.4%
Terminal rate	13/30 (43%)	13/34 (38%)	10/25 (40%)	14/27 (52%)
First incidence (days)	362	440	145	350
Life table test	P=0.155	P=0.422N	P=0.350	P=0.280
Logistic regression test	P=0.439	P=0.528	P=0.523N	P=0.417
Cochran-Armitage test	P=0.367			
Fisher exact test		P=0.579N	P=0.579N	P=0.421
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	43/50 (86%)	43/50 (86%)	40/50 (80%)
Adjusted rate	88.7%	87.8%	93.3%	90.8%
Terminal rate	25/30 (83%)	28/34 (82%)	22/25 (88%)	23/27 (85%)
First incidence (days)	362	440	145	350
Life table test	P=0.154	P=0.491N	P=0.106	P=0.305
Logistic regression test	P=0.543N	P=0.328	P=0.271	P=0.503
Cochran-Armitage test	P=0.439N			
Fisher exact test		P=0.298	P=0.298	P=0.598N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE C4
Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Northwest			
1,3-Butadiene	18/50	5/50	21/50
Allyl glycidyl ether	7/50	0/50	7/50
α-Chloroacetophenone	7/50	6/50	11/50
Epinephrine hydrochloride	11/50	5/50	15/50
Ethyl chloride	3/50	2/50	5/50
Hexachlorocyclopentadiene	11/49	0/49	11/49
o-Chlorobenzalmalononitrile	7/49	7/49	14/49
Overall Historical Incidence			
Total	113/673 (16.8%)	45/673 (6.7%)	150/673 (22.3%)
Standard deviation	7.6%	5.6%	9.0%
Range	6%-36%	0%-16%	10%-42%

[a] Data as of 31 March 1993

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Ozone[a]

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	16	10	19	20
Natural deaths	4	6	6	3
Survivors				
Died last week of study	1			
Terminal sacrifice	29	34	25	27
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(46)	(46)	(48)	(48)
Degeneration, hyaline		1 (2%)		
Intestine small, duodenum	(49)	(47)	(48)	(48)
Necrosis			1 (2%)	
Intestine small, ileum	(49)	(48)	(49)	(50)
Inflammation, chronic active		1 (2%)		
Peyer's patch, infiltration cellular, plasma cell	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Basophilic focus		2 (4%)		
Clear cell focus	4 (8%)	4 (8%)	2 (4%)	1 (2%)
Cyst	1 (2%)			
Degeneration, fatty			1 (2%)	
Eosinophilic focus	1 (2%)	2 (4%)		2 (4%)
Fibrosis	1 (2%)		1 (2%)	
Hematopoietic cell proliferation			1 (2%)	
Inflammation, chronic active		1 (2%)		
Karyomegaly		1 (2%)		
Mineralization		1 (2%)		
Necrosis	3 (6%)	1 (2%)	1 (2%)	
Centrilobular, necrosis		1 (2%)		2 (4%)
Mesentery	(4)	(5)	(1)	(3)
Inflammation, chronic active		1 (20%)		
Fat, necrosis	3 (75%)	3 (60%)		3 (100%)
Pancreas	(49)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)		
Basophilic focus		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Hyperplasia, squamous	1 (2%)	1 (2%)		
Inflammation, acute	3 (6%)	1 (2%)		
Necrosis	1 (2%)		1 (2%)	
Stomach, glandular	(50)	(49)	(50)	(50)
Inflammation, acute			1 (2%)	
Tooth	(1)	(1)	(1)	(1)
Developmental malformation				1 (100%)
Inflammation, chronic active	1 (100%)			

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

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	8 (16%)	18 (36%)	11 (22%)	17 (34%)
Inflammation, chronic active	1 (2%)			
Thrombosis	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia	14 (28%)	9 (18%)	12 (24%)	15 (30%)
Hypertrophy	26 (52%)	31 (62%)	22 (44%)	22 (44%)
Capsule, hyperplasia	3 (6%)		1 (2%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	2 (4%)	2 (4%)	3 (6%)
Pituitary gland	(47)	(49)	(49)	(48)
Cyst	1 (2%)		1 (2%)	1 (2%)
Pars distalis, hyperplasia	5 (11%)	2 (4%)	1 (2%)	
Thyroid gland	(49)	(50)	(50)	(50)
Inflammation, chronic				2 (4%)
Follicular cell, hyperplasia	6 (12%)	8 (16%)	10 (20%)	17 (34%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)	2 (4%)	1 (2%)	
Penis			(2)	(1)
Inflammation, acute			1 (50%)	1 (100%)
Preputial gland	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Hyperplasia	1 (2%)			
Inflammation, chronic active	12 (24%)	4 (8%)	6 (12%)	2 (4%)
Necrosis				1 (2%)
Prostate	(49)	(50)	(47)	(49)
Inflammation, suppurative	1 (2%)		2 (4%)	2 (4%)
Artery, inflammation, chronic active	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)	1 (2%)		1 (2%)
Testes	(50)	(50)	(50)	(50)
Atrophy	4 (8%)	2 (4%)	1 (2%)	4 (8%)
Mineralization	1 (2%)			3 (6%)
Interstitial cell, hyperplasia			1 (2%)	
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Atrophy	1 (2%)			
Necrosis	1 (2%)			

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Hematopoietic System (continued)				
Lymph node	(4)	(6)	(3)	(10)
Iliac, infiltration cellular, plasma cell	1 (25%)	1 (17%)	1 (33%)	7 (70%)
Inguinal, infiltration cellular, plasma cell		1 (17%)		
Renal, infiltration cellular, plasma cell	1 (25%)			1 (10%)
Lymph node, mesenteric	(48)	(49)	(49)	(49)
Angiectasis	1 (2%)			1 (2%)
Hemorrhage				2 (4%)
Spleen	(49)	(50)	(49)	(50)
Angiectasis			1 (2%)	
Hematopoietic cell proliferation	2 (4%)	3 (6%)	3 (6%)	6 (12%)
Necrosis	1 (2%)			
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Ulcer	1 (2%)			
Prepuce, inflammation, chronic active	8 (16%)	6 (12%)	12 (24%)	20 (40%)
Subcutaneous tissue, fibrosis				1 (2%)
Subcutaneous tissue, inflammation, granulomatous	1 (2%)	1 (2%)		
Musculoskeletal System				
None				
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Necrosis	1 (2%)			
Meninges, inflammation, chronic		1 (2%)		
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)			
Inflammation, chronic active	4 (8%)	13 (26%)	4 (8%)	9 (18%)
Artery, inflammation, chronic active	1 (2%)			
Epiglottis, hyperplasia	1 (2%)			6 (12%)
Epiglottis, metaplasia, squamous				2 (4%)
Lung	(50)	(50)	(50)	(50)
Fibrosis			1 (2%)	
Hemorrhage	1 (2%)		1 (2%)	1 (2%)
Thrombosis	1 (2%)			
Alveolar epithelium, hyperplasia	4 (8%)	6 (12%)	2 (4%)	3 (6%)
Alveolar epithelium, metaplasia			48 (96%)	50 (100%)
Alveolus, infiltration cellular, histiocyte			18 (36%)	31 (62%)
Bronchiole, erosion				1 (2%)
Bronchiole, hyperplasia				1 (2%)
Bronchiole, necrosis				3 (6%)
Bronchus, erosion				1 (2%)
Bronchus, necrosis				1 (2%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Respiratory System (continued)				
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	2 (4%)			
Lateral wall, degeneration, hyaline	2 (4%)	1 (2%)	49 (98%)	50 (100%)
Lateral wall, fibrosis			47 (94%)	49 (98%)
Lateral wall, hyperplasia			42 (84%)	50 (100%)
Lateral wall, inflammation, suppurative		8 (16%)	42 (84%)	50 (100%)
Lateral wall, metaplasia, squamous		3 (6%)	3 (6%)	36 (72%)
Nasopharyngeal duct, inflammation, chronic active		1 (2%)		
Olfactory epithelium, atrophy	1 (2%)	3 (6%)	3 (6%)	11 (22%)
Olfactory epithelium, metaplasia	1 (2%)			
Trachea	(50)	(50)	(50)	(50)
Infiltration cellular, polymorphonuclear			1 (2%)	
Special Senses System				
Eye		(1)		
Cataract		1 (100%)		
Harderian gland	(1)	(8)	(6)	(4)
Hyperplasia		1 (13%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	1 (2%)	1 (2%)	1 (2%)	
Infarct	3 (6%)	1 (2%)	1 (2%)	6 (12%)
Metaplasia, osseous		1 (2%)		4 (8%)
Nephropathy	43 (86%)	40 (80%)	46 (92%)	37 (74%)
Papilla, inflammation, suppurative	6 (12%)	3 (6%)	8 (16%)	14 (28%)
Pelvis, dilatation	3 (6%)	4 (8%)	4 (8%)	4 (8%)
Urethra				(3)
Inflammation, suppurative				1 (33%)
Bulbourethral gland, inflammation, suppurative				2 (67%)
Urinary bladder	(50)	(50)	(50)	(49)
Inflammation, chronic active	6 (12%)	3 (6%)	8 (16%)	13 (27%)

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

TABLE D1 Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Ozone 144

TABLE D2 Individual Animal Respiratory System Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Ozone 148

TABLE D3 Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Ozone 152

TABLE D4 Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Female $B6C3F_1$ Mice 158

TABLE D5 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Ozone 159

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

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			2	
Moribund	15	10	9	9
Natural deaths	6	3	6	1
Survivors				
Terminal sacrifice	29	37	33	40
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(50)	(47)	(46)	(50)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Intestine large, colon	(50)	(50)	(47)	(50)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Sarcoma stromal, metastatic, uterus	1 (2%)			
Intestine large, rectum	(49)	(43)	(45)	(45)
Intestine large, cecum	(50)	(50)	(47)	(50)
Intestine small, duodenum	(47)	(48)	(45)	(49)
Polyp adenomatous	1 (2%)			
Intestine small, jejunum	(50)	(47)	(46)	(49)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Intestine small, ileum	(50)	(49)	(45)	(50)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Hepatocellular carcinoma	14 (28%)	4 (8%)	5 (10%)	3 (6%)
Hepatocellular carcinoma, multiple	1 (2%)	1 (2%)		
Hepatocellular adenoma	13 (26%)	13 (26%)	12 (24%)	8 (16%)
Hepatocellular adenoma, multiple	7 (14%)	5 (10%)	5 (10%)	
Hepatocholangiocarcinoma	1 (2%)			
Histiocytic sarcoma		1 (2%)		1 (2%)
Osteosarcoma, metastatic, bone	1 (2%)			
Mesentery	(11)	(4)	(4)	(1)
Carcinoma, metastatic, pancreas	1 (9%)			
Hemangiosarcoma	1 (9%)			
Hepatocholangiocarcinoma, metastatic, liver	1 (9%)			
Pancreas	(49)	(50)	(48)	(50)
Carcinoma	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Salivary glands	(50)	(49)	(49)	(50)
Stomach, forestomach	(50)	(50)	(48)	(50)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Squamous cell papilloma		1 (2%)		
Stomach, glandular	(50)	(50)	(48)	(50)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Tongue				(1)
Squamous cell papilloma				1 (100%)
Tooth	(1)	(1)		
Histiocytic sarcoma		1 (100%)		
Odontoma	1 (100%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Histiocytic sarcoma		1 (2%)		
Capsule, adenoma			1 (2%)	
Capsule, carcinoma				1 (2%)
Adrenal medulla	(50)	(50)	(49)	(50)
Pheochromocytoma benign	2 (4%)	2 (4%)	3 (6%)	
Islets, pancreatic	(49)	(50)	(48)	(50)
Adenoma			1 (2%)	
Carcinoma				1 (2%)
Pituitary gland	(50)	(50)	(47)	(49)
Pars distalis, adenoma	17 (34%)	13 (26%)	14 (30%)	9 (18%)
Pars intermedia, adenoma		3 (6%)	2 (4%)	2 (4%)
Thyroid gland	(50)	(50)	(49)	(50)
Follicular cell, adenoma	2 (4%)	2 (4%)	4 (8%)	2 (4%)
Follicular cell, carcinoma	3 (6%)			
General Body System				
None				
Genital System				
Ovary	(50)	(50)	(48)	(50)
Cystadenoma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Granulosa cell tumor malignant		1 (2%)		
Granulosa cell tumor benign		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Histiocytic sarcoma		1 (2%)		
Luteoma				1 (2%)
Teratoma benign			1 (2%)	
Bilateral, cystadenoma		1 (2%)		
Uterus	(50)	(50)	(49)	(50)
Granulosa cell tumor malignant, metastatic, ovary		1 (2%)		
Hemangiosarcoma			2 (4%)	2 (4%)
Histiocytic sarcoma		1 (2%)		1 (2%)
Leiomyoma				1 (2%)
Leiomyosarcoma		1 (2%)		
Polyp stromal	1 (2%)			5 (10%)
Sarcoma stromal	2 (4%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)		3 (6%)	1 (2%)
Histiocytic sarcoma		1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Hematopoietic System (continued)				
Lymph node	(9)	(6)	(3)	(4)
Iliac, histiocytic sarcoma		1 (17%)		
Pancreatic, histiocytic sarcoma		1 (17%)		
Renal, histiocytic sarcoma		1 (17%)		
Lymph node, bronchial	(48)	(39)	(40)	(42)
Carcinoma, metastatic, pancreas	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Lymph node, mandibular	(47)	(39)	(43)	(46)
Lymph node, mesenteric	(49)	(49)	(46)	(47)
Carcinoma, metastatic, pancreas	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Histiocytic sarcoma		1 (2%)		
Lymph node, mediastinal	(41)	(42)	(39)	(35)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Histiocytic sarcoma		1 (2%)		
Spleen	(49)	(50)	(48)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	4 (8%)	2 (4%)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Thymus	(47)	(49)	(46)	(47)
Histiocytic sarcoma		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(48)	(49)
Carcinoma	1 (2%)			1 (2%)
Skin	(50)	(50)	(49)	(50)
Basal cell carcinoma			1 (2%)	
Squamous cell carcinoma	1 (2%)			
Subcutaneous tissue, hemangiosarcoma		1 (2%)	1 (2%)	1 (2%)
Subcutaneous tissue, sarcoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(49)	(50)
Osteosarcoma	1 (2%)			
Skeletal muscle	(1)		(1)	(1)
Hepatocholangiocarcinoma, metastatic, liver	1 (100%)			
Sarcoma			1 (100%)	
Nervous System				
Brain	(50)	(50)	(49)	(50)
Respiratory System				
Larynx	(50)	(50)	(49)	(50)
Lung	(50)	(50)	(49)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	5 (10%)	5 (10%)	7 (14%)
Alveolar/bronchiolar adenoma, multiple				1 (2%)
Alveolar/bronchiolar carcinoma	2 (4%)	2 (4%)	5 (10%)	8 (16%)
Carcinoma, metastatic, harderian gland	1 (2%)	1 (2%)	1 (2%)	1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Respiratory System (continued)				
Lung (continued)	(50)	(50)	(49)	(50)
Granulosa cell tumor malignant, metastatic, ovary		1 (2%)		
Hemangiosarcoma, metastatic, uterus				1 (2%)
Hepatocellular carcinoma, metastatic, liver	6 (12%)	2 (4%)	2 (4%)	2 (4%)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Histiocytic sarcoma		1 (2%)		
Squamous cell carcinoma, metastatic, skin	1 (2%)			
Mediastinum, hemangiosarcoma	1 (2%)			
Nose	(50)	(50)	(48)	(50)
Carcinoma, metastatic, harderian gland	2 (4%)			
Special Senses System				
Harderian gland	(3)	(2)	(4)	(3)
Adenoma	1 (33%)		3 (75%)	1 (33%)
Carcinoma	2 (67%)	2 (100%)	1 (25%)	2 (67%)
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Histiocytic sarcoma		1 (2%)		
Urinary bladder	(49)	(50)	(48)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		1 (2%)
Lymphoma malignant	7 (14%)	17 (34%)	14 (28%)	11 (22%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	44	42	40
Total primary neoplasms	91	79	92	74
Total animals with benign neoplasms	33	33	32	29
Total benign neoplasms	50	47	53	39
Total animals with malignant neoplasms	31	28	28	26
Total malignant neoplasms	41	32	39	35
Total animals with metastatic neoplasms	13	4	3	4
Total metastatic neoplasm	33	5	4	4

[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
Individual Animal Respiratory System Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Ozone: 0 ppm

Number of Days on Study	439	511	531	538	544	546	601	634	636	638	649	653	667	667	681	689	694	700	723	723	728	735	735	735	735
Carcass ID Number	0138	0133	0145	0143	0144	0142	0128	0147	0131	0115	0126	0120	0136	0136	0114	0149	0113	0129	0119	0124	0120	0105	0108	0109	0112
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X													X		X	
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, harderian gland				X																					
Hepatocellular carcinoma, metastatic, liver											X	X		X				X					X		
Hepatocholangiocarcinoma, metastatic, liver					X																				
Squamous cell carcinoma, metastatic, skin								X																	
Mediastinum, hemangiosarcoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, harderian gland				X											X										
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	735	735	735	735	735	736	736	736	736	736	736	736	736	736	737	737	737	737	737	737	737	737	737	737	737	
Carcass ID Number	0121	0122	0123	0134	0146	0101	0106	0101	0125	0132	0137	0140	0144	0148	0103	0107	0111	0116	0117	0118	0127	0135	0139	0141	0150	Total Tissues/ Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																							X			4
Alveolar/bronchiolar carcinoma			X												X											2
Carcinoma, metastatic, harderian gland																										1
Hepatocellular carcinoma, metastatic, liver																									X	6
Hepatocholangiocarcinoma, metastatic, liver																										1
Squamous cell carcinoma, metastatic, skin																										1
Mediastinum, hemangiosarcoma													X													1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, harderian gland																										2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

+: Tissue examined microscopically X: Lesion present A: Autolysis precludes examination

TABLE D2
Individual Animal Respiratory System Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Ozone: 0.12 ppm

Number of Days on Study	4	4	5	5	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	7	7	6	9	0	1	5	8	8	8	8	0	2	3	3	3	3	3	3	3	3	3	3	3	3
	1	1	7	4	8	2	3	1	1	1	7	3	3	5	5	5	5	5	5	5	5	5	5	5	5
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	2	4	0	1	0	2	3	0	0	4	3	1	1	0	1	1	1	2	2	3	3	4	4	4	5
	2	6	2	1	8	9	3	3	6	1	7	7	3	1	4	6	8	4	6	4	8	2	5	7	0
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X												X					
Alveolar/bronchiolar carcinoma												X													
Carcinoma, metastatic, harderian gland																									
Granulosa cell tumor malignant, metastatic, ovary																									
Hepatocellular carcinoma, metastatic, liver													X												
Histiocytic sarcoma					X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Total
	0	0	0	0	1	2	2	2	3	3	3	4	4	1	1	1	2	2	2	3	3	3	4	4	4	Tissues/
	4	5	7	9	2	1	3	7	0	1	5	3	9	0	5	9	0	5	8	2	6	9	0	4	8	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X														X										X	5
Alveolar/bronchiolar carcinoma																							X			2
Carcinoma, metastatic, harderian gland																		X								1
Granulosa cell tumor malignant, metastatic, ovary											X															1
Hepatocellular carcinoma, metastatic, liver												X														2
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Respiratory System Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Ozone: 0.5 ppm

Number of Days on Study	1	4	5	5	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	
	4	8	2	7	3	4	4	5	6	7	7	9	0	0	0	2	2	3	3	3	3	3	3	3	3	
	6	8	7	4	4	4	6	9	7	7	8	3	4	9	9	3	8	5	5	5	5	5	5	5	5	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
	5	3	2	1	2	2	4	1	0	3	1	2	2	1	1	2	2	0	1	3	3	3	4	4	4	
	0	2	9	2	5	7	7	4	4	8	8	1	8	6	9	3	0	5	3	1	6	9	1	3	6	
Respiratory System																										
Larynx	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lung	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma									X						X		X								X	
Alveolar/bronchiolar carcinoma														X			X									
Carcinoma, metastatic, harderian gland								X																		
Hepatocellular carcinoma, metastatic, liver						X																				
Nose	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	5	5	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	Total
	4	4	0	0	0	1	1	2	2	3	3	3	4	4	0	0	0	0	1	1	2	3	3	4	4	Tissues/
	8	9	2	6	7	0	7	2	6	0	4	7	0	5	1	3	8	9	1	5	4	3	5	2	4	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma	X																									5
Alveolar/bronchiolar carcinoma											X	X			X											5
Carcinoma, metastatic, harderian gland																										1
Hepatocellular carcinoma, metastatic, liver																									X	2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Respiratory System Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Ozone: 1.0 ppm

Number of Days on Study	482	488	514	516	540	569	611	667	685	688	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735
Carcass ID Number	0718	0735	0730	0708	0741	0748	0720	0740	0727	0733	0702	0704	0712	0714	0716	0717	0722	0723	0724	0726	0731	0732	0739	0742	0743
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma												X													
Alveolar/bronchiolar adenoma, multiple														X											
Alveolar/bronchiolar carcinoma		X									X		X		X								X		
Carcinoma, metastatic, harderian gland																							X		
Hemangiosarcoma, metastatic, uterus																									X
Hepatocellular carcinoma, metastatic, liver									X								X								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	735	735	735	736	736	736	736	736	736	736	736	736	736	736	736	737	737	737	737	737	737	737	737	737	737	
Carcass ID Number	0745	0747	0750	0703	0706	0707	0711	0719	0721	0728	0729	0734	0736	0737	0738	0801	0805	0809	0910	0913	0915	0925	0944	0946	0949	Total Tissues/ Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X				X	X				X		X					X								7
Alveolar/bronchiolar adenoma, multiple																										1
Alveolar/bronchiolar carcinoma			X														X		X							8
Carcinoma, metastatic, harderian gland																										1
Hemangiosarcoma, metastatic, uterus																										1
Hepatocellular carcinoma, metastatic, liver																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Ozone

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	2/50 (4%)	2/50 (4%)	3/49 (6%)	0/50 (0%)
Adjusted rate[b]	6.9%	5.4%	8.5%	0.0%
Terminal rate[c]	2/29 (7%)	2/37 (5%)	2/33 (6%)	0/40 (0%)
First incidence (days)	735 (T)	735 (T)	693	–[e]
Life table test[d]	P=0.152N	P=0.605N	P=0.559	P=0.171N
Logistic regression test[d]	P=0.175N	P=0.605N	P=0.541	P=0.171N
Cochran-Armitage test[d]	P=0.204N			
Fisher exact test[d]		P=0.691N	P=0.490	P=0.247N
Bone Marrow: Hemangiosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/49 (6%)	1/50 (2%)
Adjusted rate	3.3%	0.0%	8.4%	2.5%
Terminal rate	0/29 (0%)	0/37 (0%)	2/33 (6%)	1/40 (3%)
First incidence (days)	728	–	677	735 (T)
Life table test	P=0.463	P=0.458N	P=0.354	P=0.691N
Logistic regression test	P=0.414	P=0.473N	P=0.322	P=0.723N
Cochran-Armitage test	P=0.396			
Fisher exact test		P=0.500N	P=0.301	P=0.753N
Harderian Gland: Adenoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	3.4%	0.0%	8.0%	2.5%
Terminal rate	1/29 (3%)	0/37 (0%)	2/33 (6%)	1/40 (3%)
First incidence (days)	735 (T)	–	527	735 (T)
Life table test	P=0.456	P=0.451N	P=0.341	P=0.688N
Logistic regression test	P=0.395	P=0.451N	P=0.303	P=0.688N
Cochran-Armitage test	P=0.397			
Fisher exact test		P=0.500N	P=0.309	P=0.753N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	4/50 (8%)	3/50 (6%)
Adjusted rate	8.1%	5.4%	10.2%	6.9%
Terminal rate	1/29 (3%)	2/37 (5%)	2/33 (6%)	2/40 (5%)
First incidence (days)	538	735 (T)	527	488
Life table test	P=0.535	P=0.417N	P=0.545	P=0.575N
Logistic regression test	P=0.454	P=0.520N	P=0.504	P=0.632
Cochran-Armitage test	P=0.460			
Fisher exact test		P=0.500N	P=0.500	P=0.661N
Liver: Hepatocellular Adenoma				
Overall rate	20/50 (40%)	18/50 (36%)	17/50 (34%)	8/50 (16%)
Adjusted rate	59.9%	48.6%	43.2%	18.6%
Terminal rate	16/29 (55%)	18/37 (49%)	12/33 (36%)	6/40 (15%)
First incidence (days)	538	735 (T)	488	516
Life table test	P<0.001N	P=0.118N	P=0.200N	P<0.001N
Logistic regression test	P=0.004N	P=0.231N	P=0.297N	P=0.005N
Cochran-Armitage test	P=0.005N			
Fisher exact test		P=0.418N	P=0.339N	P=0.007N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Liver: Hepatocellular Carcinoma				
Overall rate	15/50 (30%)	5/50 (10%)	5/50 (10%)	3/50 (6%)
Adjusted rate	39.5%	11.4%	12.5%	7.3%
Terminal rate	7/29 (24%)	1/37 (3%)	2/33 (6%)	2/40 (5%)
First incidence (days)	566	567	644	685
Life table test	P=0.003N	P=0.007N	P=0.010N	P<0.001N
Logistic regression test	P=0.014N	P=0.018N	P=0.011N	P=0.002N
Cochran-Armitage test	P=0.005N			
Fisher exact test		P=0.011N	P=0.011N	P=0.002N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	27/50 (54%)	22/50 (44%)	20/50 (40%)	11/50 (22%)
Adjusted rate	68.6%	53.2%	48.5%	25.2%
Terminal rate	17/29 (59%)	18/37 (49%)	13/33 (39%)	8/40 (20%)
First incidence (days)	538	567	488	516
Life table test	P<0.001N	P=0.049N	P=0.064N	P<0.001N
Logistic regression test	P<0.001N	P=0.141N	P=0.103N	P<0.001N
Cochran-Armitage test	P<0.001N			
Fisher exact test		P=0.212N	P=0.115N	P<0.001N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	5/50 (10%)	5/49 (10%)	8/50 (16%)
Adjusted rate	12.5%	12.9%	13.4%	20.0%
Terminal rate	3/29 (10%)	4/37 (11%)	2/33 (6%)	8/40 (20%)
First incidence (days)	636	681	667	735 (T)
Life table test	P=0.233	P=0.631	P=0.573	P=0.349
Logistic regression test	P=0.153	P=0.549	P=0.515	P=0.239
Cochran-Armitage test	P=0.130			
Fisher exact test		P=0.500	P=0.487	P=0.178
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	5/49 (10%)	8/50 (16%)
Adjusted rate	6.9%	5.2%	14.1%	19.2%
Terminal rate	2/29 (7%)	1/37 (3%)	3/33 (9%)	7/40 (18%)
First incidence (days)	735 (T)	703	709	488
Life table test	P=0.025	P=0.608N	P=0.275	P=0.114
Logistic regression test	P=0.011	P=0.649N	P=0.259	P=0.053
Cochran-Armitage test	P=0.010			
Fisher exact test		P=0.691N	P=0.210	P=0.046
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	6/50 (12%)	7/50 (14%)	9/49 (18%)	16/50 (32%)
Adjusted rate	19.2%	17.7%	24.0%	38.8%
Terminal rate	5/29 (17%)	5/37 (14%)	5/33 (15%)	15/40 (38%)
First incidence (days)	636	681	667	488
Life table test	P=0.020	P=0.568N	P=0.386	P=0.074
Logistic regression test	P=0.005	P=0.571	P=0.326	P=0.022
Cochran-Armitage test	P=0.004			
Fisher exact test		P=0.500	P=0.274	P=0.014

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	17/50 (34%)	13/50 (26%)	14/47 (30%)	9/49 (18%)
Adjusted rate	48.7%	32.1%	40.1%	22.1%
Terminal rate	12/29 (41%)	10/37 (27%)	11/31 (35%)	8/39 (21%)
First incidence (days)	511	653	644	488
Life table test	P=0.025N	P=0.098N	P=0.254N	P=0.011N
Logistic regression test	P=0.067N	P=0.193N	P=0.345N	P=0.050N
Cochran-Armitage test	P=0.080N			
Fisher exact test		P=0.257N	P=0.411N	P=0.061N
Pituitary Gland (Pars Intermedia): Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/47 (4%)	2/49 (4%)
Adjusted rate	0.0%	8.1%	5.6%	5.1%
Terminal rate	0/29 (0%)	3/37 (8%)	1/31 (3%)	2/39 (5%)
First incidence (days)	–	735 (T)	677	735 (T)
Life table test	P=0.465	P=0.167	P=0.262	P=0.306
Logistic regression test	P=0.412	P=0.167	P=0.228	P=0.306
Cochran-Armitage test	P=0.382			
Fisher exact test		P=0.121	P=0.232	P=0.242
Spleen: Hemangiosarcoma				
Overall rate	1/49 (2%)	1/50 (2%)	4/48 (8%)	2/50 (4%)
Adjusted rate	3.3%	2.7%	11.3%	5.0%
Terminal rate	0/29 (0%)	1/37 (3%)	3/33 (9%)	2/40 (5%)
First incidence (days)	728	735 (T)	677	735 (T)
Life table test	P=0.364	P=0.709N	P=0.224	P=0.607
Logistic regression test	P=0.313	P=0.725N	P=0.198	P=0.575
Cochran-Armitage test	P=0.284			
Fisher exact test		P=0.747N	P=0.175	P=0.508
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	2/50 (4%)	2/50 (4%)	4/49 (8%)	2/50 (4%)
Adjusted rate	6.9%	5.4%	12.1%	5.0%
Terminal rate	2/29 (7%)	2/37 (5%)	4/33 (12%)	2/40 (5%)
First incidence (days)	735 (T)	735 (T)	735 (T)	735 (T)
Life table test	P=0.535N	P=0.605N	P=0.397	P=0.574N
Logistic regression test	P=0.535N	P=0.605N	P=0.397	P=0.574N
Cochran-Armitage test	P=0.518			
Fisher exact test		P=0.691N	P=0.329	P=0.691N
Thyroid Gland (Follicular Cell): Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	0/49 (0%)	0/50 (0%)
Adjusted rate	8.6%	0.0%	0.0%	0.0%
Terminal rate	1/29 (3%)	0/37 (0%)	0/33 (0%)	0/40 (0%)
First incidence (days)	531	–	–	–
Life table test	P=0.067N	P=0.098N	P=0.111N	P=0.092N
Logistic regression test	P=0.075N	P=0.136N	P=0.123N	P=0.129N
Cochran-Armitage test	P=0.076N			
Fisher exact test		P=0.121N	P=0.125N	P=0.121N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	5/50 (10%)	2/50 (4%)	4/49 (8%)	2/50 (4%)
Adjusted rate	15.1%	5.4%	12.1%	5.0%
Terminal rate	3/29 (10%)	2/37 (5%)	4/33 (12%)	2/40 (5%)
First incidence (days)	531	735 (T)	735 (T)	735 (T)
Life table test	P=0.200N	P=0.146N	P=0.431N	P=0.127N
Logistic regression test	P=0.266N	P=0.206N	P=0.488N	P=0.207N
Cochran-Armitage test	P=0.286N			
Fisher exact test		P=0.218N	P=0.513N	P=0.218N
Uterus: Stromal Polyp				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	5/50 (10%)
Adjusted rate	3.4%	0.0%	0.0%	11.4%
Terminal rate	1/29 (3%)	0/37 (0%)	0/33 (0%)	3/40 (8%)
First incidence (days)	735 (T)	–	–	482
Life table test	P=0.012	P=0.451N	P=0.474N	P=0.164
Logistic regression test	P=0.006	P=0.451N	P=0.474N	P=0.092
Cochran-Armitage test	P=0.007			
Fisher exact test		P=0.500N	P=0.500N	P=0.102
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	5/50 (10%)
Adjusted rate	8.3%	0.0%	0.0%	11.4%
Terminal rate	1/29 (3%)	0/37 (0%)	0/33 (0%)	3/40 (8%)
First incidence (days)	601	–	–	482
Life table test	P=0.110	P=0.098N	P=0.110N	P=0.462
Logistic regression test	P=0.078	P=0.133N	P=0.120N	P=0.324
Cochran-Armitage test	P=0.081			
Fisher exact test		P=0.121N	P=0.121N	P=0.357
All Organs: Hemangiosarcoma				
Overall rate	2/50 (4%)	1/50 (2%)	6/50 (12%)	3/50 (6%)
Adjusted rate	6.7%	2.7%	17.2%	7.5%
Terminal rate	1/29 (3%)	1/37 (3%)	5/33 (15%)	3/40 (8%)
First incidence (days)	728	735 (T)	677	735 (T)
Life table test	P=0.318	P=0.420N	P=0.182	P=0.641
Logistic regression test	P=0.264	P=0.438N	P=0.165	P=0.605
Cochran-Armitage test	P=0.220			
Fisher exact test		P=0.500N	P=0.134	P=0.500
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	1/50 (2%)	6/50 (12%)	3/50 (6%)
Adjusted rate	6.7%	2.7%	17.2%	7.5%
Terminal rate	1/29 (3%)	1/37 (3%)	5/33 (15%)	3/40 (8%)
First incidence (days)	728	735 (T)	677	735 (T)
Life table test	P=0.318	P=0.420N	P=0.182	P=0.641
Logistic regression test	P=0.264	P=0.438N	P=0.165	P=0.605
Cochran-Armitage test	P=0.220			
Fisher exact test		P=0.500N	P=0.134	P=0.500

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
All Organs: Malignant Lymphoma (Histiocytic or Lymphocytic)				
Overall	7/50 (14%)	17/50 (34%)	14/50 (28%)	11/50 (22%)
Adjusted	20.3%	38.9%	38.6%	24.9%
Terminal	4/29 (14%)	11/37 (30%)	11/33 (33%)	8/40 (20%)
First incidence (days)	636	471	704	482
Life table test	P=0.371N	P=0.070	P=0.130	P=0.407
Logistic regression test	P=0.513	P=0.017	P=0.095	P=0.200
Cochran-Armitage test	P=0.509			
Fisher exact test		P=0.017	P=0.070	P=0.218
All Organs: Malignant Lymphoma or Histiocytic Sarcoma				
Overall rate	7/50 (14%)	18/50 (36%)	14/50 (28%)	12/50 (24%)
Adjusted rate	20.3%	40.2%	38.6%	27.3%
Terminal rate	4/29 (14%)	11/37 (30%)	11/33 (33%)	9/40 (23%)
First incidence (days)	636	471	704	482
Life table test	P=0.415N	P=0.049	P=0.130	P=0.330
Logistic regression test	P=0.460	P=0.009	P=0.095	P=0.142
Cochran-Armitage test	P=0.458			
Fisher exact test		P=0.010	P=0.070	P=0.154
All Organs: Benign Neoplasms				
Overall rate	33/50 (66%)	33/50 (66%)	32/50 (64%)	29/50 (58%)
Adjusted rate	86.4%	78.5%	72.2%	62.7%
Terminal rate	24/29 (83%)	28/37 (76%)	21/33 (64%)	23/40 (58%)
First incidence (days)	511	653	488	482
Life table test	P=0.031N	P=0.109N	P=0.255N	P=0.021N
Logistic regression test	P=0.176N	P=0.372N	P=0.453N	P=0.230N
Cochran-Armitage test	P=0.201N			
Fisher exact test		P=0.583N	P=0.500N	P=0.268N
All Organs: Malignant Neoplasms				
Overall rate	31/50 (62%)	28/50 (56%)	28/50 (56%)	26/50 (52%)
Adjusted rate	65.5%	59.3%	63.5%	57.4%
Terminal rate	13/29 (45%)	18/37 (49%)	17/33 (52%)	21/40 (53%)
First incidence (days)	439	471	644	482
Life table test	P=0.081N	P=0.140N	P=0.216N	P=0.052N
Logistic regression test	P=0.221N	P=0.513N	P=0.341N	P=0.259N
Cochran-Armitage test	P=0.215N			
Fisher exact test		P=0.342N	P=0.342N	P=0.210N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/50 (94%)	44/50 (88%)	42/50 (84%)	40/50 (80%)
Adjusted rate	94.0%	89.8%	87.4%	86.8%
Terminal rate	26/29 (90%)	32/37 (86%)	27/33 (82%)	34/40 (85%)
First incidence (days)	439	471	488	482
Life table test	P=0.008N	P=0.045N	P=0.089N	P=0.002N
Logistic regression test	P=0.028N	P=0.253N	P=0.098N	P=0.036N
Cochran-Armitage test	P=0.030N			
Fisher exact test		P=0.243N	P=0.100N	P=0.036N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, liver, lung, pituitary gland, spleen, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE D4
Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Northwest			
1,3-Butadiene	4/50	0/50	4/50
Allyl glycidyl ether	0/50	0/50	0/50
α-Chloroacetophenone	4/50	3/50	6/50
Epinephrine hydrochloride	3/50	2/50	5/50
Ethyl chloride	2/49	3/49	5/49
Hexachlorocyclopentadiene	4/48	3/48	7/48
o-Chlorobenzalmalononitrile	4/50	1/50	5/50
Overall Historical Incidence			
Total	40/659 (6.1%)	19/659 (2.9%)	58/659 (8.8%)
Standard deviation	2.8%	2.5%	3.5%
Range	0%-10%	0%-6%	0%-15%

[a] Data as of 31 March 1993

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Ozone[a]

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			2	
Moribund	15	10	9	9
Natural deaths	6	3	6	1
Survivors				
Terminal sacrifice	29	37	33	40
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(50)	(47)	(46)	(50)
Inflammation, chronic active			1 (2%)	
Intestine small, duodenum	(47)	(48)	(45)	(49)
Inflammation, acute				1 (2%)
Necrosis				1 (2%)
Intestine small, jejunum	(50)	(47)	(46)	(49)
Hyperplasia, lymphoid	1 (2%)			
Inflammation, chronic active	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)		1 (2%)	1 (2%)
Basophilic focus	1 (2%)	2 (4%)	2 (4%)	
Clear cell focus	1 (2%)	1 (2%)	2 (4%)	
Cyst			1 (2%)	
Degeneration, fatty	1 (2%)		1 (2%)	
Eosinophilic focus	3 (6%)	3 (6%)	5 (10%)	4 (8%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)	1 (2%)	
Hepatodiaphragmatic nodule				1 (2%)
Necrosis	1 (2%)	2 (4%)		1 (2%)
Centrilobular, necrosis		1 (2%)		
Mesentery	(11)	(4)	(4)	(1)
Artery, inflammation, chronic active	1 (9%)			
Fat, inflammation, chronic		1 (25%)		
Fat, necrosis	7 (64%)	3 (75%)	3 (75%)	
Pancreas	(49)	(50)	(48)	(50)
Atrophy	1 (2%)	2 (4%)		1 (2%)
Basophilic focus				1 (2%)
Cyst			1 (2%)	
Hypertrophy	1 (2%)			1 (2%)
Lipomatosis				1 (2%)
Artery, inflammation, chronic active	1 (2%)			
Salivary glands	(50)	(49)	(49)	(50)
Degeneration, fatty			1 (2%)	

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

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(48)	(50)
Diverticulum		1 (2%)		
Hyperplasia, squamous		1 (2%)		3 (6%)
Inflammation, acute		1 (2%)		5 (10%)
Mineralization			1 (2%)	
Necrosis	1 (2%)			
Stomach, glandular	(50)	(50)	(48)	(50)
Mineralization	2 (4%)	8 (16%)	6 (13%)	3 (6%)
Necrosis		1 (2%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	6 (12%)	8 (16%)	5 (10%)	5 (10%)
Mineralization	1 (2%)			
Artery, inflammation, chronic active	1 (2%)			
Atrium, thrombosis	1 (2%)	1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Hematopoietic cell proliferation			1 (2%)	
Hyperplasia	1 (2%)	4 (8%)	7 (14%)	3 (6%)
Hypertrophy		1 (2%)		4 (8%)
Vacuolization cytoplasmic	1 (2%)		1 (2%)	
Adrenal medulla	(50)	(50)	(49)	(50)
Hyperplasia	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Islets, pancreatic	(49)	(50)	(48)	(50)
Hyperplasia			1 (2%)	1 (2%)
Pituitary gland	(50)	(50)	(47)	(49)
Cyst		1 (2%)		
Pars distalis, hyperplasia	11 (22%)	14 (28%)	20 (43%)	17 (35%)
Pars intermedia, hyperplasia	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Thyroid gland	(50)	(50)	(49)	(50)
Follicular cell, hyperplasia	8 (16%)	19 (38%)	21 (43%)	25 (50%)
General Body System				
None				
Genital System				
Ovary	(50)	(50)	(48)	(50)
Angiectasis	2 (4%)		2 (4%)	
Cyst	11 (22%)	12 (24%)	13 (27%)	11 (22%)
Hyperplasia, tubular			1 (2%)	
Inflammation, suppurative				1 (2%)
Thrombosis		1 (2%)	1 (2%)	

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Genital System (continued)				
Uterus	(50)	(50)	(49)	(50)
Angiectasis	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Decidual reaction	1 (2%)			
Hydrometra	4 (8%)	8 (16%)	3 (6%)	3 (6%)
Inflammation, suppurative			1 (2%)	1 (2%)
Necrosis			1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)
Myelofibrosis	1 (2%)			
Lymph node	(9)	(6)	(3)	(4)
Iliac, angiectasis	1 (11%)			
Iliac, hematopoietic cell proliferation	1 (11%)			
Iliac, hemorrhage	1 (11%)			
Iliac, infiltration cellular, plasma cell			1 (33%)	
Iliac, infiltration cellular, histiocyte	1 (11%)			
Lumbar, infiltration cellular, histiocyte	1 (11%)			
Renal, hematopoietic cell proliferation	1 (11%)			
Lymph node, bronchial	(48)	(39)	(40)	(42)
Infiltration cellular, plasma cell			1 (3%)	
Lymph node, mandibular	(47)	(39)	(43)	(46)
Hematopoietic cell proliferation	1 (2%)			
Infiltration cellular, histiocyte	1 (2%)			
Lymph node, mesenteric	(49)	(49)	(46)	(47)
Angiectasis	1 (2%)	1 (2%)		1 (2%)
Hematopoietic cell proliferation	1 (2%)			
Hemorrhage	2 (4%)			1 (2%)
Inflammation, chronic active	2 (4%)			
Lymph node, mediastinal	(41)	(42)	(39)	(35)
Infiltration cellular, plasma cell		1 (2%)		
Spleen	(49)	(50)	(48)	(50)
Hematopoietic cell proliferation	11 (22%)	6 (12%)	10 (21%)	3 (6%)
Hyperplasia, lymphoid	2 (4%)	2 (4%)		2 (4%)
Hyperplasia, mast cell	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(48)	(49)
Hyperplasia				1 (2%)
Skin	(50)	(50)	(49)	(50)
Prepuce, inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	
Musculoskeletal System				
None				

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Ozone (continued)

	0 ppm	0.12 ppm	0.5 ppm	1.0 ppm
Nervous System				
Brain	(50)	(50)	(49)	(50)
Necrosis		1 (2%)		
Meninges, inflammation, chronic	1 (2%)	1 (2%)	1 (2%)	
Spinal cord			(1)	
Inflammation, chronic			1 (100%)	
Respiratory System				
Larynx	(50)	(50)	(49)	(50)
Inflammation, chronic active	2 (4%)			1 (2%)
Epiglottis, hyperplasia				7 (14%)
Epiglottis, metaplasia, squamous				4 (8%)
Lung	(50)	(50)	(49)	(50)
Congestion, chronic	1 (2%)	1 (2%)		
Thrombosis	1 (2%)			
Alveolar epithelium, hyperplasia	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Alveolar epithelium, metaplasia			43 (88%)	49 (98%)
Alveolus, infiltration cellular, histiocyte			11 (22%)	42 (84%)
Bronchiole, hyperplasia				2 (4%)
Bronchiole, necrosis				1 (2%)
Nose	(50)	(50)	(48)	(50)
Glands, hyperplasia				1 (2%)
Lateral wall, degeneration, hyaline	5 (10%)	18 (36%)	48 (100%)	50 (100%)
Lateral wall, fibrosis		3 (6%)	46 (96%)	50 (100%)
Lateral wall, hyperplasia			42 (88%)	50 (100%)
Lateral wall, inflammation, suppurative		5 (10%)	46 (96%)	50 (100%)
Lateral wall, metaplasia, squamous	1 (2%)	1 (2%)	11 (23%)	36 (72%)
Olfactory epithelium, atrophy	4 (8%)	1 (2%)	14 (29%)	41 (82%)
Special Senses System				
Eye				(2)
Inflammation, chronic active				1 (50%)
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Amyloid deposition	1 (2%)			
Infarct	1 (2%)		1 (2%)	1 (2%)
Nephropathy	25 (50%)	33 (66%)	29 (59%)	26 (52%)
Renal tubule, necrosis		1 (2%)		

APPENDIX E
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR INHALATION STUDY OF OZONE/NNK

TABLE E1 Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK 164

TABLE E2 Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone/NNK 168

TABLE E3 Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK 174

TABLE E4 Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone/NNK 181

TABLE E1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK[a]

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Disposition Summary						
Animals initially in study	48	48	48	48	48	48
Early deaths						
Moribund	36	36	40	38	41	36
Natural deaths	4	9	2	4	3	7
Survivors						
Terminal sacrifice	8	3	6	6	4	5
Animals examined microscopically	48	48	48	48	48	48
Alimentary System						
Intestine large, rectum	(1)	(1)				
Polyp adenomatous	1 (100%)					
Intestine large, cecum			(1)	(1)	(1)	(2)
Intestine small, jejunum					(1)	
Intestine small, ileum			(1)	(1)	(1)	(1)
Carcinoma			1 (100%)			
Liver	(26)	(28)	(27)	(36)	(39)	(42)
Cholangiocarcinoma	1 (4%)					
Hepatocellular adenoma					1 (3%)	
Mesentery	(8)	(8)	(10)	(11)	(8)	(8)
Sarcoma		1 (13%)		2 (18%)		
Sarcoma, metastatic, tissue NOS		1 (13%)				
Oral mucosa		(1)	(4)			
Pharyngeal, squamous cell papilloma			2 (50%)			
Pancreas	(1)	(1)			(1)	
Tongue	(1)		(1)			
Squamous cell papilloma	1 (100%)		1 (100%)			
Cardiovascular System						
Heart	(1)	(1)	(1)	(2)		(2)
Endocrine System						
Adrenal cortex	(4)	(3)	(1)	(1)	(4)	
Adenoma		1 (33%)				
Adrenal medulla	(4)				(1)	(1)
Pheochromocytoma malignant	1 (25%)					1 (100%)
Pheochromocytoma benign	3 (75%)				1 (100%)	
Islets, pancreatic	(1)	(1)	(5)		(1)	
Adenoma			2 (40%)			
Carcinoma	1 (100%)	1 (100%)	3 (60%)		1 (100%)	
Pituitary gland	(34)	(29)	(35)	(27)	(34)	(30)
Schwannoma malignant, metastatic, tissue NOS		1 (3%)				
Pars distalis, adenoma	32 (94%)	28 (97%)	32 (91%)	23 (85%)	31 (91%)	25 (83%)
Pars distalis, carcinoma	1 (3%)					

TABLE E1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Endocrine System (continued)						
Thyroid gland	(3)	(1)	(2)		(1)	(1)
Carcinoma	1 (33%)		1 (50%)			
Bilateral, C-cell, adenoma			1 (50%)			
C-cell, adenoma						1 (100%)
C-cell, carcinoma	1 (33%)				1 (100%)	
Follicular cell, adenoma	1 (33%)					
General Body System						
Peritoneum	(32)	(30)	(24)	(31)	(32)	(26)
Tissue NOS	(1)	(5)				(1)
Sarcoma	1 (100%)	4 (80%)				
Abdominal, osteosarcoma						1 (100%)
Genital System						
Epididymis	(1)			(1)		
Preputial gland	(8)	(8)	(6)	(2)	(9)	(3)
Adenoma	1 (13%)	1 (13%)				
Carcinoma			2 (33%)			
Seminal vesicle					(1)	
Testes	(20)	(24)	(9)	(25)	(13)	(19)
Interstitial cell, adenoma	15 (75%)	20 (83%)	9 (100%)	23 (92%)	12 (92%)	19 (100%)
Hematopoietic System						
Bone marrow			(1)	(1)		
Lymph node	(9)	(10)	(7)	(10)	(26)	(17)
Lymph node, bronchial	(38)	(23)	(32)	(26)	(28)	(30)
Alveolar/bronchiolar carcinoma, metastatic, lung						1 (3%)
Squamous cell carcinoma, metastatic, lung						1 (3%)
Lymph node, mandibular	(4)	(7)	(8)	(7)	(14)	(15)
Lymph node, mesenteric	(2)	(3)	(8)	(11)	(9)	(8)
Lymph node, mediastinal	(42)	(43)	(37)	(43)	(45)	(43)
Alveolar/bronchiolar carcinoma, metastatic, lung						1 (2%)
Squamous cell carcinoma, metastatic, lung						1 (2%)
Spleen	(35)	(33)	(32)	(37)	(39)	(39)
Fibroma	1 (3%)					
Sarcoma			1 (3%)			
Thymus		(1)	(1)	(3)	(3)	(2)
Integumentary System						
Mammary gland	(4)	(2)	(5)		(5)	(4)
Fibroadenoma	1 (25%)					1 (25%)

TABLE E1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Integumentary System (continued)						
Skin	(47)	(48)	(47)	(48)	(47)	(48)
Basal cell adenoma				1 (2%)		
Keratoacanthoma	2 (4%)	4 (8%)	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Keratoacanthoma, multiple			1 (2%)			
Squamous cell carcinoma	1 (2%)					
Squamous cell papilloma		1 (2%)	1 (2%)			2 (4%)
Trichoepithelioma					1 (2%)	
Sebaceous gland, adenoma					1 (2%)	1 (2%)
Subcutaneous tissue, fibroma	2 (4%)	2 (4%)	4 (9%)		1 (2%)	1 (2%)
Subcutaneous tissue, melanoma benign			1 (2%)			
Subcutaneous tissue, skin, site of application, osteosarcoma						1 (2%)
Subcutaneous tissue, skin, site of application, sarcoma	3 (6%)	5 (10%)	4 (9%)	5 (10%)	5 (11%)	1 (2%)
Subcutaneous tissue, skin, site of application, sarcoma, multiple				1 (2%)		
Musculoskeletal System						
None						
Nervous System						
Brain		(2)			(1)	
Cranial nerve, schwannoma malignant		1 (50%)				
Respiratory System						
Lung	(48)	(48)	(48)	(48)	(48)	(48)
Alveolar/bronchiolar adenoma	3 (6%)	1 (2%)	2 (4%)	2 (4%)	11 (23%)	14 (29%)
Alveolar/bronchiolar adenoma, multiple				1 (2%)	9 (19%)	9 (19%)
Alveolar/bronchiolar carcinoma	1 (2%)				8 (17%)	9 (19%)
Alveolar/bronchiolar carcinoma, multiple						2 (4%)
Cholangiocarcinoma, metastatic, liver	1 (2%)					
Sarcoma, metastatic, skin					1 (2%)	1 (2%)
Squamous cell carcinoma					1 (2%)	2 (4%)
Mediastinum, alveolar/ bronchiolar carcinoma, metastatic, lung						1 (2%)
Nose	(47)	(48)	(48)	(48)	(48)	(46)
Respiratory epithelium, adenoma					1 (2%)	
Pleura		(2)				
Sarcoma, metastatic, tissue NOS		1 (50%)				

TABLE E1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Special Senses System						
Zymbal's gland				(3)		(1)
Carcinoma				3 (100%)		1 (100%)
Urinary System						
Kidney	(27)	(15)	(22)	(20)	(24)	(25)
Sarcoma, metastatic, tissue NOS	1 (4%)					
Transitional epithelium, carcinoma						1 (4%)
Urinary bladder		(2)	(2)	(3)	(1)	(1)
Papilloma				1 (33%)		
Systemic Lesions						
Multiple organs[b]	(48)	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	28 (58%)	25 (52%)	25 (52%)	35 (73%)	37 (77%)	40 (83%)
Mesothelioma malignant	2 (4%)	1 (2%)		1 (2%)		
Neoplasm Summary						
Total animals with primary neoplasms[c]	48	45	48	47	48	47
Total primary neoplasms	105	96	95	100	124	134
Total animals with benign neoplasms	41	39	40	37	42	37
Total benign neoplasms	63	58	58	53	71	75
Total animals with malignant neoplasms	36	34	30	41	40	43
Total malignant neoplasms	42	38	37	47	53	59
Total animals with metastatic neoplasms	2	3			1	3
Total metastatic neoplasms	2	3			1	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 E2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone/NNK: Vehicle Control/0 ppm Ozone

Number of Days on Study	3	4	4	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6
	2	6	8	1	4	4	5	5	6	6	6	7	8	8	0	1	1	1	2	2	3	3	4	5	6
	7	6	4	5	0	3	6	6	2	4	9	6	5	9	4	1	1	3	0	0	3	8	1	5	3
Carcass ID Number	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	2	2	3	0	0	1	2	0	1	2	4	3	0	1	0	4	4	1	4	1	0	3	2	3
	1	7	4	9	6	1	9	3	9	4	5	5	6	7	5	8	0	8	6	6	2	5	7	2	2
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Cholangiocarcinoma, metastatic, liver																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	6	6	7	7	8	9	9	9	0	0	0	0	0	1	2	3	3	3	3	3	3	3	3	
	4	9	2	3	1	2	7	8	0	0	4	6	9	7	4	6	6	6	6	6	6	6	6	
Carcass ID Number	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Total
	2	1	2	4	3	4	2	1	0	0	2	2	3	3	4	0	1	1	1	3	3	3	4	Tissues/
	6	8	8	7	1	4	0	0	2	3	1	9	4	5	2	4	1	3	7	0	3	8	3	Tumors
Respiratory System																								
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma																				X	X		X	3
Alveolar/bronchiolar carcinoma																							X	1
Cholangiocarcinoma, metastatic, liver										X														1
Nose	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

+: Tissue examined microscopically
A: Autolysis precludes examination
Blank: Not examined
X: Lesion present
M: Missing tissue

TABLE E2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone/NNK: Vehicle Control/0.5 ppm Ozone

Number of Days on Study	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6
	1	4	6	6	7	0	0	1	2	4	5	5	5	5	7	8	8	8	1	1	1	2	2	3	3
	7	4	0	6	8	1	1	8	9	4	0	1	5	7	9	2	9	9	2	3	7	0	5	3	5
Carcass ID Number	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	0	3	1	0	0	1	3	1	4	0	4	4	2	2	2	0	1	2	0	3	3	1	2	1	2
	1	1	1	6	2	7	5	8	2	7	8	6	9	4	6	5	6	3	3	8	3	0	1	9	2
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																							X	X	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	
	3	3	4	4	5	6	6	7	7	8	8	9	0	0	1	1	1	3	3	3	3	3	3	
	5	9	1	1	5	2	3	1	3	3	6	7	4	4	0	5	6	6	6	6	6	6	6	
Carcass ID Number	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Total
	4	3	1	4	2	1	3	4	4	0	2	3	1	3	0	2	3	0	1	2	3	4	4	Tissues/
	3	9	4	5	0	3	6	4	1	9	5	0	5	2	8	8	4	4	2	7	7	0	7	Tumors
Respiratory System																								
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma																								2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE E2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone/NNK: 0.1 mg/kg NNK/0 ppm Ozone

Number of Days on Study	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6
	2	5	8	8	8	2	2	3	4	4	5	5	6	7	8	8	9	9	0	0	0	0	0	0	0
	9	0	6	8	9	8	9	3	0	4	0	1	2	9	3	3	0	0	2	3	3	3	4	5	5
Carcass ID Number	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	1	3	3	4	3	0	1	3	3	2	4	0	0	2	4	4	1	3	2	0	1	2	2	3	4
	5	4	2	0	8	8	1	7	3	4	6	2	1	0	4	7	6	6	5	3	7	9	1	0	3
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X															X		
Alveolar/bronchiolar adenoma, multiple	X											X		X			X				X				
Alveolar/bronchiolar carcinoma																					X				
Sarcoma, metastatic, skin												X													
Squamous cell carcinoma															X										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory epithelium, adenoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	
	1	3	4	4	6	6	6	7	7	8	8	8	8	8	9	9	0	1	1	3	3	3	3	
	7	9	1	5	1	3	9	7	8	3	3	3	3	7	5	5	4	2	5	6	6	6	6	
Carcass ID Number	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	Total
	1	1	4	2	1	0	4	4	0	0	1	2	4	1	2	3	2	3	1	0	0	2	3	Tissues/
	2	3	8	7	4	7	5	1	4	6	8	6	2	9	2	9	8	1	0	5	9	3	5	Tumors
Respiratory System																								
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma	X		X	X			X	X			X				X						X	X		11
Alveolar/bronchiolar adenoma, multiple						X											X		X	X				9
Alveolar/bronchiolar carcinoma					X				X		X	X					X		X		X			8
Sarcoma, metastatic, skin																								1
Squamous cell carcinoma																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Respiratory epithelium, adenoma												X												1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE E2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone/NNK: 0.1 mg/kg NNK/0.5 ppm Ozone

Number of Days on Study	1	2	3	3	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6
	9	5	3	8	4	5	8	0	1	2	2	3	5	5	6	8	8	9	9	0	0	1	1	1	2
	2	3	3	9	0	8	7	2	5	3	9	7	0	6	0	0	9	0	2	3	5	1	4	7	4
Carcass ID Number	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	1	3	1	1	0	0	4	0	2	1	4	4	3	0	4	3	1	2	2	0	2	2	4	2	1
	3	9	2	1	5	4	8	8	1	9	5	3	4	2	7	3	6	7	0	3	8	4	1	5	5
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																		X							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pleura					+																				
Sarcoma, metastatic, tissue NOS					X																				
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	4	5	6	6	7	7	9	0	0	0	1	1	2	2	3	3	3	
	1	4	4	5	5	6	5	9	1	9	3	8	4	4	5	9	2	8	2	5	6	6	6	
Carcass ID Number	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	
	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	Total
	4	3	3	2	4	0	2	3	3	0	1	0	3	2	3	1	1	4	4	0	1	2	3	Tissues/
	0	7	8	9	6	6	2	6	5	7	0	9	0	3	2	8	4	4	2	1	7	6	1	Tumors
Respiratory System																								
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pleura																			+					2
Sarcoma, metastatic, tissue NOS																								1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	47

TABLE E2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone/NNK: 1.0 mg/kg NNK/0 ppm Ozone

Number of Days on Study	2	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6
	3	1	4	4	6	8	9	4	5	5	6	6	6	6	7	8	0	0	0	2	3	3	3	4	6
	4	6	4	4	0	1	6	9	0	2	5	7	7	9	5	3	4	4	5	0	2	5	8	1	2
Carcass ID Number	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	1	4	2	3	0	2	3	0	1	2	2	1	3	1	3	1	0	4	1	2	4	3	3	3	1
	3	7	1	6	7	6	4	4	9	3	0	8	7	2	3	1	2	0	5	9	5	1	9	8	6
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X														
Alveolar/bronchiolar adenoma, multiple																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+

Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	
	6	6	6	7	7	8	8	8	8	9	9	0	1	1	1	2	3	3	3	3	3	3	3	
	3	9	9	1	3	0	3	3	3	7	8	3	0	3	4	5	3	6	6	6	6	6	6	
Carcass ID Number	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	
	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	Total
	2	1	3	0	0	2	1	4	4	0	0	4	1	2	2	4	3	0	0	2	3	4	4	Tissues/
	5	0	2	8	3	2	7	2	8	6	9	1	4	7	4	6	0	1	5	8	5	3	4	Tumors
Respiratory System																								
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma																			X					2
Alveolar/bronchiolar adenoma, multiple																				X				1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47

TABLE E2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Ozone/NNK: 1.0 mg/kg NNK/0.5 ppm Ozone

Number of Days on Study	2	4	4	4	4	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6
	5	0	1	5	7	0	5	5	5	6	8	8	8	8	9	0	0	0	1	1	1	3	3	4	4
	3	3	6	6	0	3	7	7	8	2	0	1	2	5	7	2	3	3	0	3	7	5	5	0	0
Carcass ID Number	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	4	3	0	1	2	0	2	0	0	3	4	4	3	2	3	1	1	1	4	0	3	4	1	2
	5	7	4	6	9	6	4	8	3	2	8	5	1	6	5	5	0	1	7	0	1	1	2	6	2
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X		X	X							X			X			X	
Alveolar/bronchiolar adenoma, multiple														X			X					X			
Alveolar/bronchiolar carcinoma																								X	X
Alveolar/bronchiolar carcinoma, multiple																									
Sarcoma, metastatic, skin																									
Squamous cell carcinoma													X												
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																									X
Nose	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	
	4	5	6	6	6	7	7	8	8	8	8	8	9	0	0	0	1	2	3	3	3	3	3	
	1	5	1	9	9	1	8	3	3	3	7	7	7	0	4	5	2	5	6	6	6	6	6	
Carcass ID Number	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Total
	0	1	2	1	3	3	4	0	1	2	1	4	2	4	4	3	3	2	0	1	2	2	3	Tissues/
	9	8	1	4	7	9	6	8	3	4	2	3	0	4	8	0	2	3	7	5	7	9	3	Tumors
Respiratory System																								
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma		X			X				X				X	X	X						X		X	14
Alveolar/bronchiolar adenoma, multiple				X			X	X				X						X	X					9
Alveolar/bronchiolar carcinoma	X		X		X			X					X								X	X		9
Alveolar/bronchiolar carcinoma, multiple																			X	X				2
Sarcoma, metastatic, skin	X																							1
Squamous cell carcinoma																			X					2
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																								1
Nose	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Trachea	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46

TABLE E3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/48 (6%)	1/48 (2%)	2/48 (4%)	3/48 (6%)
Adjusted rate	37.5%	3.2%	7.7%	35.1%
Terminal rate	3/8 (38%)	0/3 (0%)	0/6 (0%)	2/6 (33%)
First incidence (days)	736 (T)	590	625	565
Life table test[d]		P=0.595	P=0.597	P=0.554
Logistic regression test[d]		P=0.442	P=0.591	P=0.627
Fisher exact test[d]		P=0.308	P=0.500	P=0.661
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	1/48 (2%)	0/48 (0%)	0/48 (0%)	0/48 (0%)
Adjusted rate	12.5%	0.0%	0.0%	0.0%
Terminal rate	1/8 (13%)	0/3 (0%)	0/6 (0%)	0/6 (0%)
First incidence (days)	736 (T)	–[e]	–	–
Life table test		P=0.695	P=0.557	P=0.557
Logistic regression test		P=0.695	P=0.557	P=0.557
Fisher exact test		P=0.500	P=0.500	P=0.500
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	3/48 (6%)	1/48 (2%)	2/48 (4%)	3/48 (6%)
Adjusted rate	37.5%	3.2%	7.7%	35.1%
Terminal rate	3/8 (38%)	0/3 (0%)	0/6 (0%)	2/6 (33%)
First incidence (days)	736 (T)	590	625	565
Life table test		P=0.595	P=0.597	P=0.554
Logistic regression test		P=0.442	P=0.591	P=0.627
Fisher exact test		P=0.308	P=0.500	P=0.661
Oral Cavity (Oral Mucosa, Tongue, Pharynx): Squamous Cell Papilloma				
Overall rate	1/48 (2%)	0/48 (0%)	3/48 (6%)	0/48 (0%)
Adjusted rate	6.3%	0.0%	16.1%	0.0%
Terminal rate	0/8 (0%)	0/3 (0%)	0/6 (0%)	0/6 (0%)
First incidence (days)	698	–	635	–
Life table test		P=0.594	P=0.221	P=0.541
Logistic regression test		P=0.573	P=0.261	P=0.516
Fisher exact test		P=0.500	P=0.308	P=0.500
Skin: Keratoacanthoma				
Overall rate	2/48 (4%)	4/48 (8%)	3/48 (6%)	2/48 (4%)
Adjusted rate	21.3%	19.1%	33.7%	4.8%
Terminal rate	1/8 (13%)	0/3 (0%)	1/6 (17%)	0/6 (0%)
First incidence (days)	717	502	704	416
Life table test		P=0.169	P=0.368	P=0.644
Logistic regression test		P=0.295	P=0.362	P=0.682
Fisher exact test		P=0.339	P=0.500	P=0.692

TABLE E3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	3/48 (6%)	20/48 (42%)	23/48 (48%)
Adjusted rate	37.5%	91.7%	88.1%
Terminal rate	3/8 (38%)	3/4 (75%)	3/5 (60%)
First incidence (days)	736 (T)	429	557
Life table test		P<0.001	P<0.001
Logistic regression test		P<0.001	P<0.001
Fisher exact test		P<0.001	P<0.001
Lung: Alveolar/bronchiolar Carcinoma			
Overall rate	1/48 (2%)	8/48 (17%)	11/48 (23%)
Adjusted rate	12.5%	62.4%	86.4%
Terminal rate	1/8 (13%)	1/4 (25%)	4/5 (80%)
First incidence (days)	736 (T)	603	640
Life table test		P=0.003	P<0.001
Logistic regression test		P=0.004	P<0.001
Fisher exact test		P=0.015	P=0.002
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	3/48 (6%)	23/48 (48%)	28/48 (58%)
Adjusted rate	37.5%	93.2%	100.0%
Terminal rate	3/8 (38%)	3/4 (75%)	5/5 (100%)
First incidence (days)	736 (T)	429	557
Life table test		P<0.001	P<0.001
Logistic regression test		P<0.001	P<0.001
Fisher exact test		P<0.001	P<0.001
Oral Cavity (Oral Mucosa, Tongue, Pharynx): Squamous Cell Papilloma			
Overall rate	1/48 (2%)	0/48 (0%)	0/48 (0%)
Adjusted rate	6.3%	0.0%	0.0%
Terminal rate	0/8 (0%)	0/4 (0%)	0/5 (0%)
First incidence (days)	698	–	–
Life table test		P=0.665	P=0.594
Logistic regression test		P=0.570	P=0.537
Fisher exact test		P=0.500	P=0.500
Skin: Keratoacanthoma			
Overall rate	2/48 (4%)	2/48 (4%)	2/48 (4%)
Adjusted rate	21.3%	20.0%	6.0%
Terminal rate	1/8 (13%)	0/4 (0%)	0/5 (0%)
First incidence (days)	717	678	562
Life table test		P=0.462	P=0.595
Logistic regression test		P=0.535	P=0.676
Fisher exact test		P=0.692	P=0.692

TABLE E3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Squamous Cell Carcinoma				
Overall rate	3/48 (6%)	5/48 (10%)	4/48 (8%)	3/48 (6%)
Adjusted rate	25.4%	26.5%	35.4%	12.1%
Terminal rate	1/8 (13%)	0/3 (0%)	1/6 (17%)	0/6 (0%)
First incidence (days)	681	502	544	416
Life table test		P=0.157	P=0.361	P=0.593
Logistic regression test		P=0.281	P=0.396	P=0.660
Fisher exact test		P=0.357	P=0.500	P=0.661
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	2/48 (4%)	2/48 (4%)	4/48 (8%)	0/48 (0%)
Adjusted rate	18.0%	10.1%	20.9%	0.0%
Terminal rate	1/8 (13%)	0/3 (0%)	0/6 (0%)	0/6 (0%)
First incidence (days)	698	529	417	–
Life table test		P=0.506	P=0.249	P=0.296
Logistic regression test		P=0.635	P=0.314	P=0.257
Fisher exact test		P=0.692	P=0.339	P=0.247
Skin (Subcutaneous Tissue): Sarcoma				
Overall rate	3/48 (6%)	5/48 (10%)	4/48 (8%)	6/48 (13%)
Adjusted rate	14.4%	41.1%	37.5%	36.8%
Terminal rate	0/8 (0%)	0/3 (0%)	1/6 (17%)	0/6 (0%)
First incidence (days)	664	537	683	234
Life table test		P=0.185	P=0.341	P=0.216
Logistic regression test		P=0.294	P=0.390	P=0.273
Fisher exact test		P=0.357	P=0.500	P=0.243
Skin (Subcutaneous Tissue): Fibroma or Sarcoma				
Overall rate	5/48 (10%)	7/48 (15%)	8/48 (17%)	6/48 (13%)
Adjusted rate	29.8%	47.1%	50.5%	36.8%
Terminal rate	1/8 (13%)	0/3 (0%)	1/6 (17%)	0/6 (0%)
First incidence (days)	664	529	417	234
Life table test		P=0.153	P=0.147	P=0.428
Logistic regression test		P=0.288	P=0.194	P=0.536
Fisher exact test		P=0.379	P=0.276	P=0.500
Tissue NOS: Sarcoma				
Overall rate	1/48 (2%)	4/48 (8%)	0/48 (0%)	0/48 (0%)
Adjusted rate	4.2%	40.6%	0.0%	0.0%
Terminal rate	0/8 (0%)	1/3 (33%)	0/6 (0%)	0/6 (0%)
First incidence (days)	663	440	–	–
Life table test		P=0.081	P=0.569	P=0.508
Logistic regression test		P=0.175	P=0.520	P=0.509
Fisher exact test		P=0.181	P=0.500	P=0.500

TABLE E3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Squamous Cell Carcinoma			
Overall rate	3/48 (6%)	3/48 (6%)	4/48 (8%)
Adjusted rate	25.4%	28.9%	11.0%
Terminal rate	1/8 (13%)	0/4 (0%)	0/5 (0%)
First incidence (days)	681	678	470
Life table test		P=0.393	P=0.400
Logistic regression test		P=0.484	P=0.504
Fisher exact test		P=0.661	P=0.500
Skin (Subcutaneous Tissue): Fibroma			
Overall rate	2/48 (4%)	1/48 (2%)	1/48 (2%)
Adjusted rate	18.0%	2.1%	11.1%
Terminal rate	1/8 (13%)	0/4 (0%)	0/5 (0%)
First incidence (days)	698	450	704
Life table test		P=0.669	P=0.648
Logistic regression test		P=0.501	P=0.596
Fisher exact test		P=0.500	P=0.500
Skin (Subcutaneous Tissue): Sarcoma			
Overall rate	3/48 (6%)	5/48 (10%)	1/48 (2%)
Adjusted rate	14.4%	36.5%	4.3%
Terminal rate	0/8 (0%)	0/4 (0%)	0/5 (0%)
First incidence (days)	664	528	641
Life table test		P=0.195	P=0.392
Logistic regression test		P=0.341	P=0.333
Fisher exact test		P=0.357	P=0.308
Skin (Subcutaneous Tissue): Fibroma or Sarcoma			
Overall rate	5/48 (10%)	6/48 (13%)	2/48 (4%)
Adjusted rate	29.8%	37.9%	15.0%
Terminal rate	1/8 (13%)	0/4 (0%)	0/5 (0%)
First incidence (days)	664	450	641
Life table test		P=0.257	P=0.365
Logistic regression test		P=0.487	P=0.269
Fisher exact test		P=0.500	P=0.218
Tissue NOS: Sarcoma			
Overall rate	1/48 (2%)	0/48 (0%)	0/48 (0%)
Adjusted rate	4.2%	0.0%	0.0%
Terminal rate	0/8 (0%)	0/4 (0%)	0/5 (0%)
First incidence (days)	663	–	–
Life table test		P=0.557	P=0.536
Logistic regression test		P=0.529	P=0.514
Fisher exact test		P=0.500	P=0.500

TABLE E3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone
Zymbal's Gland: Carcinoma				
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	3/48 (6%)
Adjusted rate	0.0%	0.0%	0.0%	10.8%
Terminal rate	0/8 (0%)	0/3 (0%)	0/6 (0%)	0/6 (0%)
First incidence (days)	–	–	–	481
Life table test		–	–	P=0.112
Logistic regression test		–	–	P=0.136
Fisher exact test		–	–	P=0.121
All Organs: Mononuclear Cell Leukemia				
Overall rate	28/48 (58%)	25/48 (52%)	25/48 (52%)	35/48 (73%)
Adjusted rate	89.4%	92.7%	82.5%	96.5%
Terminal rate	6/8 (75%)	2/3 (67%)	3/6 (50%)	5/6 (83%)
First incidence (days)	327	389	460	444
Life table test		P=0.205	P=0.449	P=0.101
Logistic regression test		P=0.416	P=0.337	P=0.087
Fisher exact test		P=0.341	P=0.341	P=0.098
All Organs: Benign Neoplasms				
Overall rate	41/48 (85%)	39/48 (81%)	40/48 (83%)	37/48 (77%)
Adjusted rate	97.5%	100.0%	100.0%	100.0%
Terminal rate	7/8 (88%)	3/3 (100%)	6/6 (100%)	6/6 (100%)
First incidence (days)	484	389	417	416
Life table test		P=0.082	P=0.237	P=0.534
Logistic regression test		P=0.452	P=0.593	P=0.370
Fisher exact test		P=0.392	P=0.500	P=0.217
All Organs: Malignant Neoplasms				
Overall rate	36/48 (75%)	34/48 (71%)	30/48 (63%)	41/48 (85%)
Adjusted rate	93.0%	95.7%	88.4%	97.5%
Terminal rate	6/8 (75%)	2/3 (67%)	3/6 (50%)	5/6 (83%)
First incidence (days)	327	253	460	234
Life table test		P=0.126	P=0.530	P=0.163
Logistic regression test		P=0.432	P=0.142	P=0.156
Fisher exact test		P=0.409	P=0.135	P=0.153
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/48 (100%)	45/48 (94%)	48/48 (100%)	47/48 (98%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	8/8 (100%)	3/3 (100%)	6/6 (100%)	6/6 (100%)
First incidence (days)	327	253	417	234
Life table test		P=0.087	P=0.185	P=0.374
Logistic regression test		P=0.371	–[f]	P=0.609
Fisher exact test		P=0.121	P=1.000	P=0.500

TABLE E3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Zymbal's Gland: Carcinoma			
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	10.0%
Terminal rate	0/8 (0%)	0/4 (0%)	0/5 (0%)
First incidence (days)	–	–	700
Life table test		–	P=0.419
Logistic regression test		–	P=0.461
Fisher exact test		–	P=0.500
All Organs: Mononuclear Cell Leukemia			
Overall rate	28/48 (58%)	37/48 (77%)	40/48 (83%)
Adjusted rate	89.4%	100.0%	100.0%
Terminal rate	6/8 (75%)	4/4 (100%)	5/5 (100%)
First incidence (days)	327	486	403
Life table test		P=0.008	P=0.014
Logistic regression test		P=0.033	P=0.006
Fisher exact test		P=0.040	P=0.006
All Organs: Benign Neoplasms			
Overall rate	41/48 (85%)	42/48 (88%)	37/48 (77%)
Adjusted rate	97.5%	100.0%	96.8%
Terminal rate	7/8 (88%)	4/4 (100%)	4/5 (80%)
First incidence (days)	484	429	456
Life table test		P=0.048	P=0.375
Logistic regression test		P=0.369	P=0.304
Fisher exact test		P=0.500	P=0.217
All Organs: Malignant Neoplasms			
Overall rate	36/48 (75%)	40/48 (83%)	43/48 (90%)
Adjusted rate	93.0%	100.0%	100.0%
Terminal rate	6/8 (75%)	4/4 (100%)	5/5 (100%)
First incidence (days)	327	486	403
Life table test		P=0.030	P=0.055
Logistic regression test		P=0.190	P=0.044
Fisher exact test		P=0.226	P=0.053

TABLE E3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
All Organs: Benign or Malignant Neoplasms			
Overall rate	48/48 (100%)	48/48 (100%)	47/48 (98%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	8/8 (100%)	4/4 (100%)	5/5 (100%)
First incidence (days)	327	429	403
Life table test		P=0.061	P=0.228
Logistic regression test		-	P=0.500
Fisher exact test		P=1.000	P=0.500

(T)Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for lung; for other tissues, denominator is number of animals necropsied.

[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Fisher exact test compares directly the overall incidence rates.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE E4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone/NNK[a]

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Disposition Summary						
Animals initially in study	48	48	48	48	48	48
Early deaths						
Moribund	36	36	40	38	41	36
Natural deaths	4	9	2	4	3	7
Survivors						
Terminal sacrifice	8	3	6	6	4	5
Animals examined microscopically	48	48	48	48	48	48
Alimentary System						
Intestine large, colon	(1)	(1)			(1)	
Mineralization	1 (100%)	1 (100%)				
Lymphoid tissue, hyperplasia, lymphoid					1 (100%)	
Intestine large, rectum	(1)	(1)				
Mineralization		1 (100%)				
Intestine large, cecum			(1)	(1)	(1)	(2)
Ulcer					1 (100%)	1 (50%)
Intestine small, ileum			(1)	(1)	(1)	(1)
Hyperplasia, lymphoid					1 (100%)	
Peyer's patch, hyperplasia, lymphoid				1 (100%)		
Liver	(26)	(28)	(27)	(36)	(39)	(42)
Angiectasis		3 (11%)	1 (4%)	3 (8%)		5 (12%)
Degeneration, cystic	5 (19%)	4 (14%)	3 (11%)	8 (22%)	7 (18%)	17 (40%)
Hepatodiaphragmatic nodule	4 (15%)	1 (4%)	2 (7%)	3 (8%)	1 (3%)	5 (12%)
Hyperplasia						2 (5%)
Hyperplasia, focal	1 (4%)	1 (4%)	1 (4%)		4 (10%)	7 (17%)
Infiltration cellular, mixed cell	1 (4%)					1 (2%)
Inflammation				1 (3%)		
Necrosis	1 (4%)					
Necrosis, focal		1 (4%)			3 (8%)	3 (7%)
Vacuolization cytoplasmic	1 (4%)	4 (14%)	4 (15%)	4 (11%)	2 (5%)	2 (5%)
Vacuolization cytoplasmic, focal	1 (4%)		1 (4%)			1 (2%)
Bile duct, hyperplasia	1 (4%)	1 (4%)	2 (7%)			1 (2%)
Centrilobular, necrosis			1 (4%)			1 (2%)
Mesentery	(8)	(8)	(10)	(11)	(8)	(8)
Fat, necrosis	7 (88%)	5 (63%)	9 (90%)	7 (64%)	6 (75%)	8 (100%)
Oral mucosa		(1)	(4)			
Pharyngeal, foreign body		1 (100%)				
Pharyngeal, hyperplasia, squamous			2 (50%)			
Pancreas	(1)	(1)			(1)	
Artery, inflammation, chronic					1 (100%)	
Stomach, forestomach	(1)	(2)		(1)	(1)	(2)
Hyperplasia, squamous						2 (100%)
Inflammation, chronic active				1 (100%)		1 (50%)
Mineralization	1 (100%)	1 (50%)				
Ulcer		1 (50%)				

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

TABLE E4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Alimentary System (continued)						
Stomach, glandular	(1)	(3)	(1)	(1)	(3)	
Inflammation, chronic active				1 (100%)		
Mineralization	1 (100%)	2 (67%)				
Necrosis		1 (33%)			1 (33%)	
Tooth				(1)		(1)
Developmental malformation				1 (100%)		1 (100%)
Cardiovascular System						
Blood vessel	(2)	(1)				
Inflammation, chronic		1 (100%)				
Mineralization		1 (100%)				
Aorta, mineralization	2 (100%)					
Heart	(1)	(1)	(1)	(2)		(2)
Cardiomyopathy		1 (100%)				
Fibrosis						1 (50%)
Mineralization	1 (100%)					
Necrosis			1 (100%)			
Atrium, thrombosis						1 (50%)
Pericardium, fibrosis				1 (50%)		
Endocrine System						
Adrenal cortex	(4)	(3)	(1)	(1)	(4)	
Angiectasis		1 (33%)				
Vacuolization cytoplasmic					1 (25%)	
Adrenal medulla	(4)				(1)	(1)
Hemorrhage	1 (25%)					
Parathyroid gland	(2)	(1)		(1)		(1)
Hyperplasia	2 (100%)	1 (100%)		1 (100%)		1 (100%)
Pituitary gland	(34)	(29)	(35)	(27)	(34)	(30)
Pars distalis, hemorrhage						1 (3%)
Pars distalis, hyperplasia	1 (3%)		1 (3%)		2 (6%)	2 (7%)
Thyroid gland	(3)	(1)	(2)		(1)	(1)
Follicular cell, hyperplasia		1 (100%)				
General Body System						
Peritoneum	(32)	(30)	(24)	(31)	(32)	(26)
Inflammation, chronic	30 (94%)	30 (100%)	24 (100%)	30 (97%)	32 (100%)	26 (100%)
Tissue NOS	(1)	(5)				(1)
Mediastinum, inflammation, chronic active, diffuse		1 (20%)				
Genital System						
Epididymis	(1)			(1)		
Granuloma sperm	1 (100%)					

TABLE E4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Genital System (continued)						
Penis		(3)	(4)	(1)	(5)	(2)
Calculus gross observation		1 (33%)				
Calculus microscopic observation only		1 (33%)		1 (100%)		
Edema						2 (100%)
Inflammation, chronic active			2 (50%)		2 (40%)	
Preputial gland	(8)	(8)	(6)	(2)	(9)	(3)
Inflammation, chronic active	6 (75%)	5 (63%)	2 (33%)	1 (50%)	7 (78%)	3 (100%)
Inflammation, suppurative	1 (13%)					
Prostate		(1)	(2)	(2)		
Inflammation, suppurative		1 (100%)	2 (100%)	2 (100%)		
Testes	(20)	(24)	(9)	(25)	(13)	(19)
Atrophy	6 (30%)	1 (4%)	1 (11%)	5 (20%)	3 (23%)	1 (5%)
Necrosis		1 (4%)				
Interstitial cell, hyperplasia	2 (10%)					
Hematopoietic System						
Lymph node	(9)	(10)	(7)	(10)	(26)	(17)
Hyperplasia, lymphoid		1 (10%)				
Infiltration cellular, plasma cell	1 (11%)					
Pigmentation		1 (10%)				
Iliac, infiltration cellular, plasma cell	1 (11%)					1 (6%)
Iliac, pigmentation						1 (6%)
Pancreatic, hyperplasia, lymphoid				1 (10%)		
Pancreatic, inflammation, granulomatous	1 (11%)				1 (4%)	
Renal, hemorrhage	1 (11%)	1 (10%)		1 (10%)	3 (12%)	
Renal, hyperplasia, lymphoid				1 (10%)		
Renal, infiltration cellular, plasma cell	4 (44%)		1 (14%)	1 (10%)	5 (19%)	1 (6%)
Renal, inflammation, granulomatous	1 (11%)	1 (10%)	2 (29%)		1 (4%)	1 (6%)
Lymph node, bronchial	(38)	(23)	(32)	(26)	(28)	(30)
Hemorrhage	1 (3%)	1 (4%)	1 (3%)	2 (8%)		
Hyperplasia, lymphoid	2 (5%)	1 (4%)				
Necrosis			1 (3%)			
Lymph node, mandibular	(4)	(7)	(8)	(7)	(14)	(15)
Hyperplasia, lymphoid						1 (7%)
Infiltration cellular, plasma cell		5 (71%)		1 (14%)	3 (21%)	1 (7%)
Necrosis					1 (7%)	
Lymph node, mesenteric	(2)	(3)	(8)	(11)	(9)	(8)
Hyperplasia, lymphoid		1 (33%)				
Inflammation, granulomatous			1 (13%)	1 (9%)		

TABLE E4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Hematopoietic System (continued)						
Lymph node, mediastinal	(42)	(43)	(37)	(43)	(45)	(43)
Fibrosis					1 (2%)	
Hemorrhage	2 (5%)	3 (7%)	1 (3%)	2 (5%)	2 (4%)	
Infiltration cellular, plasma cell	2 (5%)	2 (5%)	1 (3%)		1 (2%)	1 (2%)
Inflammation, chronic active	1 (2%)	1 (2%)				
Necrosis			1 (3%)		1 (2%)	
Spleen	(35)	(33)	(32)	(37)	(39)	(39)
Accessory spleen	1 (3%)			1 (3%)	4 (10%)	1 (3%)
Atrophy					1 (3%)	
Congestion	2 (6%)	2 (6%)		1 (3%)		
Fibrosis	12 (34%)	7 (21%)	14 (44%)	12 (32%)	10 (26%)	13 (33%)
Hematopoietic cell proliferation	1 (3%)	1 (3%)	1 (3%)		1 (3%)	1 (3%)
Hemorrhage			2 (6%)			
Necrosis		1 (3%)	1 (3%)		3 (8%)	1 (3%)
Capsule, fibrosis						1 (3%)
Integumentary System						
Mammary gland	(4)	(2)	(5)		(5)	(4)
Galactocele	2 (50%)	2 (100%)	3 (60%)		4 (80%)	2 (50%)
Hyperplasia	1 (25%)		2 (40%)		1 (20%)	1 (25%)
Inflammation, chronic			1 (20%)		1 (20%)	
Skin	(47)	(48)	(47)	(48)	(47)	(48)
Hyperkeratosis			2 (4%)	1 (2%)	3 (6%)	2 (4%)
Hyperplasia				1 (2%)		
Inflammation, chronic	1 (2%)		1 (2%)	1 (2%)	2 (4%)	1 (2%)
Inflammation, suppurative		1 (2%)			1 (2%)	
Ulcer			2 (4%)		1 (2%)	
Dermis, cyst				1 (2%)		1 (2%)
Prepuce, inflammation, suppurative		3 (6%)	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Sebaceous gland, hyperplasia				1 (2%)		
Subcutaneous tissue, inflammation, chronic	1 (2%)					1 (2%)
Subcutaneous tissue, necrosis						1 (2%)
Subcutaneous tissue, skin, site of application, inflammation, chronic	46 (98%)	48 (100%)	47 (100%)	47 (98%)	47 (100%)	48 (100%)
Musculoskeletal System						
None						
Nervous System						
Brain		(2)			(1)	
Hemorrhage					1 (100%)	

TABLE E4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Respiratory System						
Larynx	(48)	(48)	(48)	(48)	(48)	(48)
Foreign body	3 (6%)	2 (4%)	2 (4%)			
Inflammation, suppurative	1 (2%)	3 (6%)	4 (8%)	3 (6%)		6 (13%)
Metaplasia, squamous	1 (2%)					
Mineralization		1 (2%)				1 (2%)
Lung	(48)	(48)	(48)	(48)	(48)	(48)
Congestion	2 (4%)		2 (4%)	5 (10%)	3 (6%)	
Edema		1 (2%)		3 (6%)		1 (2%)
Fibrosis, focal	2 (4%)		1 (2%)		1 (2%)	
Hemorrhage	3 (6%)	2 (4%)	2 (4%)	1 (2%)	5 (10%)	3 (6%)
Mineralization	4 (8%)	2 (4%)				
Alveolar epithelium, hyperplasia		1 (2%)				
Alveolar epithelium, hyperplasia, atypical			10 (21%)	12 (25%)	39 (81%)	33 (69%)
Alveolar epithelium, metaplasia		35 (73%)		47 (98%)		45 (94%)
Alveolus, infiltration cellular, focal, histiocyte			1 (2%)		1 (2%)	
Alveolus, infiltration cellular, histiocyte	1 (2%)	7 (15%)	1 (2%)	9 (19%)	8 (17%)	13 (27%)
Bronchus, inflammation, suppurative		1 (2%)				
Interstitium, fibrosis		34 (71%)		46 (96%)		45 (94%)
Interstitium, inflammation, chronic, diffuse	3 (6%)	2 (4%)	1 (2%)			
Perivascular, inflammation, chronic	1 (2%)					
Serosa, fibrosis					1 (2%)	
Serosa, inflammation, chronic active		1 (2%)				
Nose	(47)	(48)	(48)	(48)	(48)	(46)
Inflammation, chronic			1 (2%)			
Inflammation, suppurative	1 (2%)	3 (6%)	1 (2%)	3 (6%)	5 (10%)	1 (2%)
Thrombosis				1 (2%)		
Goblet cell, lateral wall, hyperplasia	3 (6%)	38 (79%)		45 (94%)	3 (6%)	42 (91%)
Lateral wall, hyperplasia	5 (11%)	46 (96%)	4 (8%)	48 (100%)	5 (10%)	46 (100%)
Nasopharyngeal duct, infiltration cellular, mixed cell					1 (2%)	
Olfactory epithelium, degeneration, hyaline	47 (100%)	47 (98%)	48 (100%)	48 (100%)	45 (94%)	46 (100%)
Olfactory epithelium, metaplasia	1 (2%)	1 (2%)	4 (8%)	1 (2%)	2 (4%)	
Turbinate, necrosis		1 (2%)		1 (2%)		

TABLE E4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Ozone/NNK (continued)

	Vehicle Control	Vehicle Control/ 0.5 ppm Ozone	0.1 mg/kg NNK/ 0 ppm Ozone	0.1 mg/kg NNK/ 0.5 ppm Ozone	1.0 mg/kg NNK/ 0 ppm Ozone	1.0 mg/kg NNK/ 0.5 ppm Ozone
Special Senses System						
Eye	(3)	(1)			(1)	(4)
Cataract	3 (100%)	1 (100%)			1 (100%)	2 (50%)
Hemorrhage	1 (33%)					1 (25%)
Ciliary body, retina, degeneration	2 (67%)	1 (100%)				2 (50%)
Urinary System						
Kidney	(27)	(15)	(22)	(20)	(24)	(25)
Cyst	1 (4%)	2 (13%)				1 (4%)
Hydronephrosis	1 (4%)					
Infarct				1 (5%)	2 (8%)	1 (4%)
Nephropathy	27 (100%)	14 (93%)	22 (100%)	18 (90%)	22 (92%)	22 (88%)
Pigmentation, hemosiderin				1 (5%)		
Urinary bladder		(2)	(2)	(3)	(1)	(1)
Calculus gross observation		1 (50%)				
Calculus microscopic observation only		1 (50%)				1 (100%)
Hemorrhage		1 (50%)		2 (67%)	1 (100%)	
Inflammation, chronic active			1 (50%)			
Inflammation, suppurative					1 (100%)	
Transitional epithelium, necrosis				1 (33%)		

APPENDIX F
SUMMARY OF LESIONS IN MALE RATS IN THE LIFETIME INHALATION STUDY OF OZONE

TABLE F1 Summary of the Incidence of Neoplasms in Male Rats in the Lifetime Inhalation Study of Ozone 188

TABLE F2 Individual Animal Respiratory System Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Ozone 192

TABLE F3 Statistical Analysis of Primary Neoplasms in Male Rats in the Lifetime Inhalation Study of Ozone 195

TABLE F4 Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Ozone 200

TABLE F1
Summary of the Incidence of Neoplasms in Male Rats in the Lifetime Inhalation Study of Ozone[a]

	0 ppm	0.5 ppm	1.0 ppm
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	47	43	42
Natural deaths	3	7	7
Survivors			
Terminal sacrifice			1
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, colon	(49)	(50)	(49)
Polyp adenomatous	1 (2%)		
Sarcoma, metastatic, uncertain primary site		1 (2%)	
Intestine large, cecum	(49)	(50)	(49)
Intestine small, duodenum	(50)	(48)	(49)
Intestine small, jejunum	(50)	(46)	(46)
Carcinoma			1 (2%)
Intestine small, ileum	(49)	(47)	(48)
Liver	(50)	(50)	(50)
Hepatocellular adenoma	1 (2%)	1 (2%)	2 (4%)
Histiocytic sarcoma			1 (2%)
Sarcoma, metastatic, uncertain primary site		1 (2%)	
Mesentery	(16)	(17)	(7)
Sarcoma, metastatic, uncertain primary site		1 (6%)	
Schwannoma malignant	1 (6%)		
Thymoma malignant, metastatic, thymus		1 (6%)	
Oral mucosa			(4)
Pharyngeal, squamous cell carcinoma			1 (25%)
Pharyngeal, squamous cell papilloma			2 (50%)
Pancreas	(50)	(50)	(50)
Adenoma	2 (4%)		2 (4%)
Histiocytic sarcoma			1 (2%)
Sarcoma, metastatic, uncertain primary site		1 (2%)	
Schwannoma malignant, metastatic, mesentery	1 (2%)		
Salivary glands	(50)	(49)	(50)
Stomach, forestomach	(50)	(50)	(50)
Sarcoma, metastatic, uncertain primary site		1 (2%)	
Stomach, glandular	(50)	(50)	(50)
Sarcoma, metastatic, uncertain primary site		1 (2%)	
Tongue	(1)		
Hemangiosarcoma	1 (100%)		
Cardiovascular System			
Heart	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Histiocytic sarcoma			1 (2%)
Osteosarcoma, metastatic, bone		1 (2%)	
Thymoma malignant, metastatic, thymus		1 (2%)	

TABLE F1
Summary of the Incidence of Neoplasms in Male Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Endocrine System			
Adrenal cortex	(50)	(49)	(50)
Adenoma	1 (2%)		1 (2%)
Adrenal medulla	(50)	(49)	(50)
Pheochromocytoma malignant	2 (4%)	1 (2%)	
Pheochromocytoma complex		1 (2%)	
Pheochromocytoma benign	13 (26%)	11 (22%)	15 (30%)
Bilateral, pheochromocytoma benign	15 (30%)	7 (14%)	8 (16%)
Islets, pancreatic	(50)	(50)	(50)
Adenoma	6 (12%)	2 (4%)	4 (8%)
Carcinoma	5 (10%)	2 (4%)	1 (2%)
Parathyroid gland	(49)	(50)	(50)
Adenoma			1 (2%)
Pituitary gland	(50)	(48)	(50)
Pars distalis, adenoma	36 (72%)	30 (63%)	34 (68%)
Thyroid gland	(50)	(50)	(50)
Bilateral, C-cell, adenoma			1 (2%)
C-cell, adenoma	5 (10%)	8 (16%)	5 (10%)
C-cell, carcinoma	1 (2%)	1 (2%)	
Follicular cell, adenoma	1 (2%)		
Follicular cell, carcinoma	1 (2%)		1 (2%)
General Body System			
Peritoneum		(1)	
Genital System			
Epididymis	(50)	(50)	(50)
Sarcoma, metastatic, uncertain primary site		1 (2%)	
Schwannoma malignant, metastatic, mesentery	1 (2%)		
Penis	(4)	(4)	(1)
Preputial gland	(50)	(48)	(50)
Adenoma	1 (2%)		1 (2%)
Carcinoma		3 (6%)	2 (4%)
Prostate	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)
Seminal vesicle	(50)	(50)	(50)
Schwannoma malignant, metastatic, mesentery	1 (2%)		
Testes	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	18 (36%)	27 (54%)	27 (54%)
Interstitial cell, adenoma	17 (34%)	6 (12%)	6 (12%)
Hematopoietic System			
Bone marrow	(50)	(50)	(50)
Lymph node	(20)	(13)	(13)
Lymph node, bronchial	(36)	(37)	(36)
Lymph node, mandibular	(48)	(47)	(47)
Hemangiosarcoma, metastatic, tongue	1 (2%)		
Lymph node, mesenteric	(49)	(49)	(49)
Schwannoma malignant, metastatic, mesentery	1 (2%)		

TABLE F1
Summary of the Incidence of Neoplasms in Male Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Hematopoietic System (continued)			
Lymph node, mediastinal	(45)	(48)	(47)
Histiocytic sarcoma			1 (2%)
Sarcoma, metastatic, uncertain primary site		1 (2%)	
Spleen	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)
Sarcoma	1 (2%)		
Sarcoma, metastatic, uncertain primary site		1 (2%)	
Thymus	(46)	(42)	(46)
Thymoma malignant		1 (2%)	
Integumentary System			
Mammary gland	(31)	(21)	(26)
Skin	(50)	(50)	(49)
Keratoacanthoma	4 (8%)	2 (4%)	4 (8%)
Squamous cell carcinoma			1 (2%)
Squamous cell papilloma		1 (2%)	
Subcutaneous tissue, fibroma		1 (2%)	1 (2%)
Subcutaneous tissue, fibroma, multiple		1 (2%)	
Subcutaneous tissue, melanoma benign			1 (2%)
Musculoskeletal System			
Bone	(50)	(50)	(50)
Osteosarcoma	1 (2%)	1 (2%)	
Skeletal muscle	(1)	(3)	(2)
Histiocytic sarcoma			1 (50%)
Sarcoma, metastatic, uncertain primary site		1 (33%)	
Schwannoma malignant, metastatic, mesentery	1 (100%)		
Nervous System			
Brain	(50)	(50)	(50)
Astrocytoma benign		1 (2%)	
Astrocytoma malignant	1 (2%)		1 (2%)
Meninges, granular cell tumor benign	1 (2%)		
Respiratory System			
Larynx	(50)	(48)	(47)
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	3 (6%)	
Alveolar/bronchiolar carcinoma		1 (2%)	
Carcinoma, metastatic, thyroid gland		1 (2%)	
Histiocytic sarcoma			1 (2%)
Osteosarcoma, metastatic, bone	1 (2%)	1 (2%)	
Thymoma malignant, metastatic, thymus		1 (2%)	
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Nose	(50)	(49)	(49)

TABLE F1
Summary of the Incidence of Neoplasms in Male Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Respiratory System (continued)			
Pleura		(1)	
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (100%)	
Special Senses System			
Zymbal's gland			(1)
Carcinoma			1 (100%)
Urinary System			
Kidney	(50)	(50)	(50)
Liposarcoma	1 (2%)		
Oncocytoma benign	1 (2%)		
Sarcoma, metastatic, uncertain primary site		1 (2%)	
Renal tubule, adenoma	3 (6%)	2 (4%)	2 (4%)
Urinary bladder	(50)	(50)	(49)
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)
Leukemia mononuclear	29 (58%)	23 (46%)	29 (58%)
Mesothelioma malignant	2 (4%)	4 (8%)	1 (2%)
Neoplasm Summary			
Total animals with primary neoplasms[c]	50	48	49
Total primary neoplasms	174	142	158
Total animals with benign neoplasms	49	46	48
Total benign neoplasms	128	104	118
Total animals with malignant neoplasms	35	30	33
Total malignant neoplasms	46	38	40
Total animals with metastatic neoplasms	3	5	
Total metastatic neoplasms	7	20	
Total animals with malignant neoplasms uncertain primary site		1	
Total uncertain neoplasms		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 F2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Ozone: 0 ppm

Number of Days on Study	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6
	6	6	8	8	8	2	3	4	8	8	8	8	8	8	8	9	9	9	9	0	1	1	2	2	3
	7	8	1	4	5	1	9	4	0	0	0	3	5	5	6	1	1	8	9	0	0	5	1	5	2
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	5	2	4	4	2	5	4	5	0	3	5	3	0	2	1	0	1	3	4	4	0	4	0	0	2
	0	6	9	6	0	2	8	3	8	8	4	5	2	5	3	3	8	9	1	0	1	2	6	9	4
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Osteosarcoma, metastatic, bone								X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	8	
	3	5	6	6	6	6	6	6	7	7	7	8	8	9	0	0	0	1	2	3	6	6	9	9	3	
	6	1	1	3	3	3	6	9	0	4	4	1	3	1	5	5	8	8	2	3	1	6	3	7	0	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	Total
	5	4	1	0	1	1	2	1	2	0	1	3	3	2	0	4	1	3	2	3	2	4	5	1	1	Tissues/
	1	4	9	5	0	7	3	2	9	7	6	3	2	7	4	5	4	7	1	0	8	3	6	5	1	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																	X			X						2
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

+: Tissue examined microscopically
A: Autolysis precludes examination

Blank: Not examined

X: Lesion present
I: Insufficient tissue

TABLE F2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Ozone: 0.5 ppm

Number of Days on Study	219	345	346	384	401	411	419	433	439	495	515	516	521	524	525	531	548	571	581	581	586	601	605	607	608
Carcass ID Number	1027	1023	1054	1006	1003	1051	1006	1033	1037	1035	1012	1028	1014	1040	1019	1037	1036	1021	1044	1045	1010	1022	1009	1024	1043
Respiratory System																									
Larynx	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																				X					
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, thyroid gland																									
Osteosarcoma, metastatic, bone																									
Thymoma malignant, metastatic, thymus		X																							
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																									
Nose	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pleura																									
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	621	623	628	636	642	648	650	663	666	670	674	681	684	695	699	701	705	719	719	722	733	735	755	757	847	
Carcass ID Number	1030	1007	1042	1008	1029	1005	1002	1056	1020	1055	1015	1026	1032	1052	1048	1050	1041	1131	1135	1353	1418	1406	1401	1373	1039	Total Tissues/ Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma														X				X								3
Alveolar/bronchiolar carcinoma						X																				1
Carcinoma, metastatic, thyroid gland		X																								1
Osteosarcoma, metastatic, bone								X																		1
Thymoma malignant, metastatic, thymus																										1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung						X																				1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pleura						+																				1
Alveolar/bronchiolar carcinoma, metastatic, lung						X																				1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE F2
Individual Animal Respiratory System Tumor Pathology of Male Rats in the Lifetime Inhalation Study of Ozone: 1.0 ppm

Number of Days on Study	3	3	4	4	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6
	4	8	1	5	8	2	3	3	4	5	6	6	7	8	0	1	1	1	1	2	3	4	5	5	6
	2	3	9	3	8	4	6	9	9	7	7	9	9	1	8	4	6	8	9	1	2	3	0	3	3
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	0	3	1	3	5	2	1	1	3	1	3	0	2	2	2	0	2	2	5	5	0	3	1	5	4
	1	3	7	2	5	9	1	3	7	4	4	9	7	2	8	6	1	0	2	1	7	8	5	3	4
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	A	+	+	+	I	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	8	8	8	
	6	7	9	0	0	0	0	1	2	2	3	3	4	4	5	5	5	5	6	6	6	7	1	2	7	
	6	1	1	1	5	5	8	6	8	8	7	7	1	7	1	4	6	6	0	1	1	9	7	5	4	
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Total
	0	4	3	1	0	5	3	3	4	4	1	1	3	4	1	1	2	3	2	2	5	4	4	4	2	Tissues/
	2	5	0	8	4	6	6	1	1	3	2	9	5	8	6	0	5	9	3	4	4	6	2	0	6	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma										X																1
Nose	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE F3
Statistical Analysis of Primary Neoplasms in Male Rats in the Lifetime Inhalation Study of Ozone

	0 ppm	0.5 ppm	1.0 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rate[a]	28/50 (56%)	18/49 (37%)	23/50 (46%)
Adjusted rate[b]	100.0%	100.0%	100.0%
Terminal rate[c]	0/0 (0%)	0/0 (0%)	1/1 (100%)
First incidence (days)	580	601	383
Life table test[d]	P=0.010N	P=0.176N	P=0.023N
Logistic regression test[d]	P=0.039N	P=0.095N	P=0.078N
Cochran-Armitage test[d]	P=0.183N		
Fisher exact test[d]		P=0.042N	P=0.212N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma			
Overall rate	28/50 (56%)	19/49 (39%)	23/50 (46%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	0/0 (0%)	0/0 (0%)	1/1 (100%)
First incidence (days)	580	495	383
Life table test	P=0.010N	P=0.223N	P=0.023N
Logistic regression test	P=0.046N	P=0.157N	P=0.078N
Cochran-Armitage test	P=0.184N		
Fisher exact test		P=0.065N	P=0.212N
Kidney (Renal Tubule): Adenoma			
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	14.5%	25.0%	55.6%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	580	719	756
Life table test	P=0.200N	P=0.533N	P=0.336N
Logistic regression test	P=0.301N	P=0.563N	P=0.418N
Cochran-Armitage test	P=0.406N		
Fisher exact test		P=0.500N	P=0.500N
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate	25.9%	22.3%	0.0%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	708	581	–[e]
Life table test	P=0.075N	P=0.475	P=0.085N
Logistic regression test	P=0.161N	P=0.427	P=0.169N
Cochran-Armitage test	P=0.202N		
Fisher exact test		P=0.500	P=0.247N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rate	25.9%	26.2%	0.0%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	708	581	–
Life table test	P=0.091N	P=0.307	P=0.085N
Logistic regression test	P=0.182N	P=0.266	P=0.169N
Cochran-Armitage test	P=0.222N		
Fisher exact test		P=0.339	P=0.247N

TABLE F3
Statistical Analysis of Primary Neoplasms in Male Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Oral Cavity (Oral Mucosa): Squamous Cell Papilloma or Squamous Cell Carcinoma			
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	100.0%
Terminal rate	0/0 (0%)	0/0 (0%)	1/1 (100%)
First incidence (days)	–	–	619
Life table test	P=0.127	–	P=0.285
Logistic regression test	P=0.064	–	P=0.167
Cochran-Armitage test	P=0.037		
Fisher exact test		–	P=0.121
Pancreatic Islets: Adenoma			
Overall rate	6/50 (12%)	2/50 (4%)	4/50 (8%)
Adjusted rate	26.6%	10.2%	17.9%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	468	623	653
Life table test	P=0.165N	P=0.178N	P=0.206N
Logistic regression test	P=0.275N	P=0.145N	P=0.368N
Cochran-Armitage test	P=0.290N		
Fisher exact test		P=0.134N	P=0.370N
Pancreatic Islets: Carcinoma			
Overall rate	5/50 (10%)	2/50 (4%)	1/50 (2%)
Adjusted rate	58.1%	37.8%	3.6%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	580	674	650
Life table test	P=0.038N	P=0.355N	P=0.072N
Logistic regression test	P=0.049N	P=0.274N	P=0.098N
Cochran-Armitage test	P=0.060N		
Fisher exact test		P=0.218N	P=0.102N
Pancreatic Islets: Adenoma or Carcinoma			
Overall rate	11/50 (22%)	4/50 (8%)	5/50 (10%)
Adjusted rate	69.2%	44.1%	20.9%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	468	623	650
Life table test	P=0.022N	P=0.116N	P=0.036N
Logistic regression test	P=0.043N	P=0.067N	P=0.083N
Cochran-Armitage test	P=0.053N		
Fisher exact test		P=0.045N	P=0.086N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	36/50 (72%)	30/48 (63%)	34/50 (68%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	0/0 (0%)	0/0 (0%)	1/1 (100%)
First incidence (days)	467	346	383
Life table test	P=0.048N	P=0.464N	P=0.068N
Logistic regression test	P=0.336N	P=0.316N	P=0.385N
Cochran-Armitage test	P=0.374N		
Fisher exact test		P=0.216N	P=0.414N

TABLE F3
Statistical Analysis of Primary Neoplasms in Male Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Preputial Gland: Carcinoma			
Overall rate	0/50 (0%)	3/48 (6%)	2/50 (4%)
Adjusted rate	0.0%	20.8%	19.2%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	–	411	751
Life table test	P=0.374	P=0.098	P=0.400
Logistic regression test	P=0.206	P=0.149	P=0.342
Cochran-Armitage test	P=0.203		
Fisher exact test		P=0.114	P=0.247
Preputial Gland: Adenoma or Carcinoma			
Overall rate	1/50 (2%)	3/48 (6%)	3/50 (6%)
Adjusted rate	2.6%	20.8%	30.7%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	583	411	751
Life table test	P=0.413	P=0.247	P=0.456
Logistic regression test	P=0.251	P=0.369	P=0.393
Cochran-Armitage test	P=0.240		
Fisher exact test		P=0.293	P=0.309
Skin: Keratoacanthoma			
Overall rate	4/50 (8%)	2/50 (4%)	4/50 (8%)
Adjusted rate	24.7%	28.4%	19.6%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	610	636	632
Life table test	P=0.277N	P=0.416N	P=0.351N
Logistic regression test	P=0.502N	P=0.401N	P=0.591N
Cochran-Armitage test	P=0.579		
Fisher exact test		P=0.339N	P=0.643N
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma			
Overall rate	4/50 (8%)	2/50 (4%)	5/50 (10%)
Adjusted rate	24.7%	28.4%	59.8%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	610	636	632
Life table test	P=0.385N	P=0.416N	P=0.449N
Logistic regression test	P=0.538	P=0.401N	P=0.604
Cochran-Armitage test	P=0.424		
Fisher exact test		P=0.339N	P=0.500
Testes: Adenoma			
Overall rate	35/50 (70%)	33/50 (66%)	33/50 (66%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	0/0 (0%)	0/0 (0%)	1/1 (100%)
First incidence (days)	468	411	419
Life table test	P=0.030N	P=0.410	P=0.045N
Logistic regression test	P=0.265N	P=0.382	P=0.283N
Cochran-Armitage test	P=0.375N		
Fisher exact test		P=0.415N	P=0.415N

TABLE F3
Statistical Analysis of Primary Neoplasms in Male Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Thyroid Gland (C-cell): Adenoma			
Overall rate	5/50 (10%)	8/50 (16%)	6/50 (12%)
Adjusted rate	50.8%	54.5%	59.0%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	610	531	383
Life table test	P=0.403N	P=0.187	P=0.565N
Logistic regression test	P=0.470	P=0.188	P=0.502
Cochran-Armitage test	P=0.440		
Fisher exact test		P=0.277	P=0.500
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rate	6/50 (12%)	9/50 (18%)	6/50 (12%)
Adjusted rate	52.1%	56.4%	59.0%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	583	531	383
Life table test	P=0.311N	P=0.191	P=0.450N
Logistic regression test	P=0.533N	P=0.200	P=0.617
Cochran-Armitage test	P=0.557		
Fisher exact test		P=0.288	P=0.620N
All Organs: Mononuclear Cell Leukemia			
Overall rate	29/50 (58%)	23/50 (46%)	29/50 (58%)
Adjusted rate	100.0%	100.0%	92.7%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	481	411	419
Life table test	P=0.123N	P=0.404N	P=0.174N
Logistic regression test	P=0.462	P=0.287N	P=0.453
Cochran-Armitage test	P=0.540		
Fisher exact test		P=0.158N	P=0.580N
All Organs: Malignant Mesothelioma			
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)
Adjusted rate	5.6%	13.6%	10.0%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	539	433	754
Life table test	P=0.346N	P=0.264	P=0.407N
Logistic regression test	P=0.414N	P=0.435	P=0.504N
Cochran-Armitage test	P=0.406N		
Fisher exact test		P=0.339	P=0.500N
All Organs: Benign Neoplasms			
Overall rate	49/50 (98%)	46/50 (92%)	48/50 (96%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	0/0 (0%)	0/0 (0%)	1/1 (100%)
First incidence (days)	467	346	383
Life table test	P=0.045N	P=0.379	P=0.066N
Logistic regression test	P=0.588N	P=0.689	P=0.632N
Cochran-Armitage test	P=0.406N		
Fisher exact test		P=0.181N	P=0.500N

TABLE F3
Statistical Analysis of Primary Neoplasms in Male Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
All Organs: Malignant Neoplasms			
Overall rate	35/50 (70%)	31/50 (62%)	33/50 (66%)
Adjusted rate	100.0%	100.0%	94.7%
Terminal rate	0/0 (0%)	0/0 (0%)	0/1 (0%)
First incidence (days)	468	345	419
Life table test	P=0.062N	P=0.517	P=0.090N
Logistic regression test	P=0.323N	P=0.387N	P=0.371N
Cochran-Armitage test	P=0.376N		
Fisher exact test		P=0.263N	P=0.415N
All Organs: Benign or Malignant Neoplasms			
Overall rate	50/50 (100%)	48/50 (96%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	0/0 (0%)	0/0 (0%)	1/1 (100%)
First incidence (days)	467	345	383
Life table test	P=0.048N	P=0.334	P=0.070N
Logistic regression test	P=0.623N	P=0.990N	–[f]
Cochran-Armitage test	P=0.360N		
Fisher exact test		P=0.247N	P=0.500N

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, kidney, lung, pancreas, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE F4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Ozone[a]

	0 ppm	0.5 ppm	1.0 ppm
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	47	43	42
Natural deaths	3	7	7
Survivors			
Terminal sacrifice			1
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, colon	(49)	(50)	(49)
Mineralization	5 (10%)		
Parasite metazoan	2 (4%)	2 (4%)	3 (6%)
Intestine large, rectum	(50)	(50)	(49)
Parasite metazoan		3 (6%)	2 (4%)
Intestine large, cecum	(49)	(50)	(49)
Inflammation, acute	1 (2%)	1 (2%)	1 (2%)
Mineralization	2 (4%)		
Parasite metazoan	3 (6%)	5 (10%)	4 (8%)
Artery, inflammation, chronic active		1 (2%)	
Intestine small, duodenum	(50)	(48)	(49)
Inflammation, acute		2 (4%)	
Intestine small, ileum	(49)	(47)	(48)
Inflammation, acute	1 (2%)		
Mineralization	3 (6%)		
Liver	(50)	(50)	(50)
Angiectasis	2 (4%)	6 (12%)	3 (6%)
Basophilic focus	23 (46%)	18 (36%)	23 (46%)
Clear cell focus	1 (2%)		
Degeneration, cystic	15 (30%)	13 (26%)	16 (32%)
Degeneration, fatty	14 (28%)	13 (26%)	9 (18%)
Eosinophilic focus	1 (2%)	1 (2%)	5 (10%)
Hepatodiaphragmatic nodule	3 (6%)	2 (4%)	6 (12%)
Infiltration cellular, mixed cell		1 (2%)	
Mixed cell focus	3 (6%)	2 (4%)	1 (2%)
Necrosis	1 (2%)	4 (8%)	3 (6%)
Thrombosis	2 (4%)		1 (2%)
Vacuolization cytoplasmic, focal	1 (2%)	2 (4%)	
Bile duct, hyperplasia	39 (78%)	38 (76%)	29 (58%)
Centrilobular, necrosis	7 (14%)	10 (20%)	6 (12%)
Mesentery	(16)	(17)	(7)
Inflammation, chronic active	1 (6%)		
Artery, inflammation, chronic active		1 (6%)	
Artery, mineralization	6 (38%)	3 (18%)	
Fat, hemorrhage			1 (14%)
Fat, necrosis	8 (50%)	10 (59%)	4 (57%)
Oral mucosa			(4)
Gingival, hyperplasia, squamous			1 (25%)

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

TABLE F4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Alimentary System (continued)			
Pancreas	(50)	(50)	(50)
Atrophy	28 (56%)	28 (56%)	28 (56%)
Basophilic focus		1 (2%)	2 (4%)
Hyperplasia	3 (6%)	3 (6%)	
Inflammation, suppurative		1 (2%)	
Thrombosis	1 (2%)		
Artery, inflammation	1 (2%)		
Artery, mineralization	2 (4%)		
Salivary glands	(50)	(49)	(50)
Artery, mineralization	1 (2%)		
Duct, metaplasia, squamous			1 (2%)
Stomach, forestomach	(50)	(50)	(50)
Diverticulum		1 (2%)	1 (2%)
Hyperplasia, squamous		1 (2%)	2 (4%)
Mineralization	7 (14%)	2 (4%)	
Necrosis	9 (18%)	10 (20%)	5 (10%)
Stomach, glandular	(50)	(50)	(50)
Inflammation, acute		1 (2%)	
Mineralization	13 (26%)	7 (14%)	6 (12%)
Necrosis	6 (12%)	4 (8%)	2 (4%)
Cardiovascular System			
Blood vessel	(9)	(4)	(4)
Aorta, mineralization	9 (100%)	4 (100%)	4 (100%)
Heart	(50)	(50)	(50)
Cardiomyopathy	40 (80%)	44 (88%)	39 (78%)
Inflammation, chronic active		1 (2%)	
Mineralization	1 (2%)		
Artery, mineralization	8 (16%)	6 (12%)	2 (4%)
Atrium, thrombosis	7 (14%)	3 (6%)	1 (2%)
Endocrine System			
Adrenal cortex	(50)	(49)	(50)
Atrophy	1 (2%)	2 (4%)	
Hyperplasia	18 (36%)	22 (45%)	25 (50%)
Hypertrophy	7 (14%)	5 (10%)	1 (2%)
Necrosis		1 (2%)	1 (2%)
Vacuolization cytoplasmic	5 (10%)	2 (4%)	4 (8%)
Adrenal medulla	(50)	(49)	(50)
Hyperplasia	21 (42%)	19 (39%)	14 (28%)
Islets, pancreatic	(50)	(50)	(50)
Hyperplasia	2 (4%)	4 (8%)	3 (6%)
Parathyroid gland	(49)	(50)	(50)
Hyperplasia	15 (31%)	10 (20%)	12 (24%)
Pituitary gland	(50)	(48)	(50)
Hemorrhage			1 (2%)
Mineralization	1 (2%)		1 (2%)
Thrombosis	1 (2%)	1 (2%)	
Pars distalis, hyperplasia	7 (14%)	9 (19%)	8 (16%)
Pars distalis, necrosis		1 (2%)	

TABLE F4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Ozone *(continued)*

	0 ppm	0.5 ppm	1.0 ppm
Endocrine System (continued)			
Thyroid gland	(50)	(50)	(50)
C-cell, hyperplasia	28 (56%)	29 (58%)	22 (44%)
Follicular cell, hyperplasia	1 (2%)	2 (4%)	3 (6%)
General Body System			
None			
Genital System			
Epididymis	(50)	(50)	(50)
Granuloma sperm	2 (4%)		1 (2%)
Penis	(4)	(4)	(1)
Inflammation, acute	1 (25%)	2 (50%)	
Preputial gland	(50)	(48)	(50)
Inflammation, chronic active	5 (10%)	2 (4%)	6 (12%)
Prostate	(50)	(50)	(50)
Hyperplasia	6 (12%)	1 (2%)	4 (8%)
Inflammation, chronic active	10 (20%)	3 (6%)	4 (8%)
Seminal vesicle	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)		
Mineralization	3 (6%)	2 (4%)	
Testes	(50)	(50)	(50)
Atrophy	9 (18%)	5 (10%)	1 (2%)
Artery, inflammation, chronic active	4 (8%)	3 (6%)	3 (6%)
Artery, mineralization	1 (2%)		
Interstitial cell, hyperplasia	10 (20%)	4 (8%)	10 (20%)
Hematopoietic System			
Lymph node	(20)	(13)	(13)
Iliac, infiltration cellular, plasma cell			1 (8%)
Lumbar, hemorrhage		1 (8%)	
Renal, hemorrhage	7 (35%)	4 (31%)	
Renal, pigmentation		1 (8%)	
Lymph node, bronchial	(36)	(37)	(36)
Hemorrhage		1 (3%)	
Lymph node, mandibular	(48)	(47)	(47)
Hemorrhage		1 (2%)	1 (2%)
Infiltration cellular, plasma cell	3 (6%)		
Necrosis			1 (2%)
Lymph node, mesenteric	(49)	(49)	(49)
Ectasia	1 (2%)		
Lymph node, mediastinal	(45)	(48)	(47)
Hemorrhage		2 (4%)	
Spleen	(50)	(50)	(50)
Fibrosis	16 (32%)	6 (12%)	13 (26%)
Hematopoietic cell proliferation		1 (2%)	
Hemorrhage	1 (2%)	1 (2%)	3 (6%)
Necrosis	1 (2%)	1 (2%)	2 (4%)

TABLE F4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Integumentary System			
Mammary gland	(31)	(21)	(26)
Galactocele	4 (13%)		1 (4%)
Hyperplasia, atypical	1 (3%)		
Inflammation, chronic active	1 (3%)		
Skin	(50)	(50)	(49)
Cyst			1 (2%)
Fibrosis	1 (2%)		
Hyperkeratosis	4 (8%)		
Inflammation, chronic active	16 (32%)	3 (6%)	5 (10%)
Prepuce, inflammation, acute		1 (2%)	
Musculoskeletal System			
Bone	(50)	(50)	(50)
Fibrous osteodystrophy	15 (30%)	8 (16%)	5 (10%)
Hyperostosis		1 (2%)	
Nervous System			
Brain	(50)	(50)	(50)
Hemorrhage			2 (4%)
Mineralization		1 (2%)	1 (2%)
Necrosis		1 (2%)	1 (2%)
Pigmentation, hemosiderin	1 (2%)		
Meninges, hyperplasia			1 (2%)
Respiratory System			
Larynx	(50)	(48)	(47)
Mineralization	5 (10%)	1 (2%)	
Epiglottis, metaplasia, squamous		20 (42%)	43 (91%)
Lung	(50)	(50)	(50)
Congestion, chronic	1 (2%)	1 (2%)	
Foreign body		1 (2%)	
Hemorrhage	2 (4%)	2 (4%)	3 (6%)
Inflammation, chronic active	3 (6%)		1 (2%)
Inflammation, suppurative		1 (2%)	1 (2%)
Mineralization	10 (20%)	6 (12%)	4 (8%)
Necrosis	1 (2%)		
Thrombosis	1 (2%)		1 (2%)
Alveolar epithelium, hyperplasia	4 (8%)	4 (8%)	6 (12%)
Alveolar epithelium, metaplasia		45 (90%)	50 (100%)
Alveolus, infiltration cellular, histiocyte		38 (76%)	49 (98%)
Artery, mediastinum, mineralization	7 (14%)	1 (2%)	1 (2%)
Artery, mediastinum, thrombosis	1 (2%)		
Interstitium, fibrosis		44 (88%)	50 (100%)

TABLE F4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Respiratory System (continued)			
Nose	(50)	(49)	(49)
Inflammation, suppurative	14 (28%)	13 (27%)	18 (37%)
Thrombosis	11 (22%)	6 (12%)	3 (6%)
Goblet cell, lateral wall, hyperplasia	1 (2%)	46 (94%)	48 (98%)
Lateral wall, hyperplasia	10 (20%)	48 (98%)	47 (96%)
Lateral wall, metaplasia, squamous	10 (20%)	23 (47%)	40 (82%)
Olfactory epithelium, degeneration, hyaline	49 (98%)	49 (100%)	49 (100%)
Olfactory epithelium, metaplasia	5 (10%)	1 (2%)	
Trachea	(50)	(50)	(50)
Mineralization	3 (6%)		2 (4%)
Special Senses System			
Eye	(1)	(2)	(3)
Cataract		1 (50%)	2 (67%)
Degeneration	1 (100%)	1 (50%)	2 (67%)
Retina, atrophy		1 (50%)	1 (33%)
Harderian gland			(1)
Inflammation, chronic active			1 (100%)
Urinary System			
Kidney	(50)	(50)	(50)
Cyst	3 (6%)	3 (6%)	3 (6%)
Hyperplasia, oncocytic	1 (2%)		
Mineralization	10 (20%)	5 (10%)	2 (4%)
Nephropathy	50 (100%)	49 (98%)	50 (100%)
Pelvis, dilatation	1 (2%)		
Pelvis, inflammation, acute	1 (2%)		
Renal tubule, hyperplasia	6 (12%)	4 (8%)	3 (6%)
Renal tubule, hyperplasia, oncocytic	1 (2%)	1 (2%)	
Transitional epithelium, hyperplasia	2 (4%)		
Urinary bladder	(50)	(50)	(49)
Hemorrhage	1 (2%)		
Infiltration cellular, polymorphonuclear		1 (2%)	1 (2%)
Inflammation, chronic active	2 (4%)		
Transitional epithelium, hyperplasia	2 (4%)		

APPENDIX G
SUMMARY OF LESIONS IN FEMALE RATS IN THE LIFETIME INHALATION STUDY OF OZONE

TABLE G1 Summary of the Incidence of Neoplasms in Female Rats in the Lifetime Inhalation Study of Ozone 206

TABLE G2 Individual Animal Respiratory System Tumor Pathology of Female Rats in the Lifetime Inhalation Study of Ozone 210

TABLE G3 Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Ozone 213

TABLE G4 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Lifetime Inhalation Study of Ozone 218

TABLE G1
Summary of the Incidence of Neoplasms in Female Rats in the Lifetime Inhalation Study of Ozone[a]

	0 ppm	0.5 ppm	1.0 ppm
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	36	37	40
Natural deaths	8	7	3
Survivors			
Terminal sacrifice	6	6	7
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, colon	(49)	(50)	(50)
Intestine large, rectum	(48)	(50)	(47)
Polyp adenomatous		1 (2%)	
Intestine large, cecum	(48)	(49)	(50)
Intestine small, duodenum	(47)	(49)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Intestine small, jejunum	(47)	(47)	(49)
Intestine small, ileum	(46)	(47)	(49)
Liver	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)	
Histiocytic sarcoma		1 (2%)	
Mesentery	(11)	(7)	(4)
Oral mucosa	(2)	(2)	
Gingival, squamous cell carcinoma	1 (50%)		
Pharyngeal, squamous cell papilloma		2 (100%)	
Pancreas	(49)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Salivary glands	(50)	(50)	(50)
Stomach, forestomach	(49)	(50)	(50)
Stomach, glandular	(49)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)	
Tongue		(1)	(1)
Squamous cell carcinoma			1 (100%)
Cardiovascular System			
Heart	(50)	(50)	(50)
Endocrine System			
Adrenal cortex	(50)	(49)	(50)
Adenoma		1 (2%)	1 (2%)
Carcinoma	2 (4%)		
Adrenal medulla	(50)	(49)	(50)
Pheochromocytoma malignant	1 (2%)		1 (2%)
Pheochromocytoma complex		1 (2%)	
Pheochromocytoma benign	12 (24%)	14 (29%)	12 (24%)
Bilateral, pheochromocytoma benign	1 (2%)	1 (2%)	2 (4%)

TABLE G1
Summary of the Incidence of Neoplasms in Female Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Endocrine System (continued)			
Islets, pancreatic	(49)	(49)	(50)
Carcinoma	2 (4%)	1 (2%)	1 (2%)
Parathyroid gland	(49)	(48)	(44)
Adenoma		1 (2%)	
Pituitary gland	(50)	(49)	(50)
Carcinoma		1 (2%)	
Pars distalis, adenoma	44 (88%)	40 (82%)	37 (74%)
Pars intermedia, adenoma		1 (2%)	
Thyroid gland	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Bilateral, C-cell, adenoma			1 (2%)
C-cell, adenoma	6 (12%)	6 (12%)	3 (6%)
C-cell, adenoma, multiple	1 (2%)		
C-cell, carcinoma	4 (8%)	3 (6%)	4 (8%)
Follicular cell, carcinoma		1 (2%)	1 (2%)
General Body System			
None			
Genital System			
Clitoral gland	(45)	(48)	(47)
Adenoma	3 (7%)	3 (6%)	6 (13%)
Carcinoma		2 (4%)	3 (6%)
Ovary	(50)	(50)	(50)
Granulosa cell tumor benign		1 (2%)	
Uterus	(50)	(50)	(50)
Carcinoma	1 (2%)		
Polyp stromal	5 (10%)	3 (6%)	6 (12%)
Polyp stromal, multiple	1 (2%)		
Sarcoma stromal			1 (2%)
Schwannoma malignant	1 (2%)		1 (2%)
Hematopoietic System			
Bone marrow	(50)	(49)	(50)
Histiocytic sarcoma		1 (2%)	
Lymph node	(10)	(12)	(9)
Lymph node, bronchial	(27)	(42)	(34)
Lymph node, mandibular	(47)	(44)	(46)
Histiocytic sarcoma		1 (2%)	
Lymph node, mesenteric	(47)	(50)	(49)
Histiocytic sarcoma		1 (2%)	
Lymph node, mediastinal	(42)	(45)	(45)
Histiocytic sarcoma		1 (2%)	
Spleen	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	
Sarcoma			1 (2%)
Thymus	(44)	(47)	(48)

TABLE G1
Summary of the Incidence of Neoplasms in Female Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Integumentary System			
Mammary gland	(50)	(50)	(50)
Adenoma, multiple			1 (2%)
Carcinoma	7 (14%)	5 (10%)	7 (14%)
Carcinoma, multiple	1 (2%)		
Fibroadenoma	9 (18%)	18 (36%)	20 (40%)
Fibroadenoma, multiple	10 (20%)	10 (20%)	5 (10%)
Skin	(49)	(50)	(50)
Keratoacanthoma	1 (2%)		
Schwannoma malignant, metastatic, uterus	1 (2%)		
Squamous cell carcinoma	1 (2%)		1 (2%)
Squamous cell papilloma	1 (2%)		1 (2%)
Subcutaneous tissue, fibroma	1 (2%)	1 (2%)	2 (4%)
Subcutaneous tissue, fibrosarcoma	1 (2%)		1 (2%)
Subcutaneous tissue, fibrous histiocytoma		1 (2%)	
Subcutaneous tissue, melanoma malignant			1 (2%)
Musculoskeletal System			
Bone	(50)	(50)	(50)
Osteoma			1 (2%)
Nervous System			
Brain	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland		1 (2%)	
Glioma benign	1 (2%)		
Spinal cord	(1)		
Respiratory System			
Larynx	(49)	(47)	(50)
Carcinoma, metastatic, thyroid gland			1 (2%)
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)	1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)	
Carcinoma, metastatic, mammary gland	1 (2%)		
Carcinoma, metastatic, thyroid gland			2 (4%)
Carcinoma, metastatic, adrenal cortex	1 (2%)		
Histiocytic sarcoma		1 (2%)	
Squamous cell carcinoma	1 (2%)		
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)	1 (2%)	
Nose	(50)	(49)	(50)
Chondroma			1 (2%)
Special Senses System			
Zymbal's gland		(1)	
Adenoma		1 (100%)	

TABLE G1
Summary of the Incidence of Neoplasms in Female Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Urinary System			
Kidney	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Histiocytic sarcoma	1 (2%)		
Renal tubule, adenoma		1 (2%)	1 (2%)
Renal tubule, carcinoma	1 (2%)		
Urinary bladder	(50)	(49)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)	
Transitional epithelium, carcinoma		1 (2%)	
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	
Leukemia mononuclear	21 (42%)	22 (44%)	20 (40%)
Neoplasm Summary			
Total animals with primary neoplasms[c]	49	48	50
Total primary neoplasms	143	146	145
Total animals with benign neoplasms	47	45	48
Total benign neoplasms	96	106	101
Total animals with malignant neoplasms	34	31	32
Total malignant neoplasms	47	40	44
Total animals with metastatic neoplasms	4	2	2
Total metastatic neoplasms	4	9	3

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

TABLE G2
Individual Animal Respiratory System Tumor Pathology of Female Rats in the Lifetime Inhalation Study of Ozone: 0 ppm

Number of Days on Study	0	4	4	4	4	4	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6
	3	0	1	3	6	9	5	5	5	6	7	8	8	0	2	3	3	3	4	4	5	5	7	8	9
	1	6	7	4	7	4	4	6	7	6	7	1	1	4	7	1	5	5	6	9	2	9	8	4	1
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
	3	1	2	3	3	4	0	4	1	3	5	1	2	2	0	2	3	4	2	5	0	5	5	0	1
	6	7	3	3	2	9	4	6	6	5	4	0	5	0	5	1	8	7	2	0	9	2	3	6	2
Respiratory System																										
Larynx	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																										
Carcinoma, metastatic, mammary gland			X																							
Carcinoma, metastatic, adrenal cortex										X																
Squamous cell carcinoma																X										
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	8	8	8	8	8	8	8	8	
	9	9	0	1	1	2	2	3	3	4	4	4	5	6	8	8	9	2	4	7	7	7	7	7	7	
	1	6	8	8	9	2	3	1	1	7	7	8	8	0	3	9	0	7	2	4	4	4	4	4	4	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	Total
	5	1	1	1	0	4	2	2	4	0	4	3	5	2	4	1	0	4	3	0	1	3	3	4	5	Tissues/
	6	4	1	3	3	5	8	4	0	7	1	9	5	9	8	5	2	3	4	8	9	0	1	2	1	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																		X								1
Carcinoma, metastatic, mammary gland																										1
Carcinoma, metastatic, adrenal cortex																										1
Squamous cell carcinoma																										1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																		X								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

+: Tissue examined microscopically
A: Autolysis precludes examination
X: Lesion present
I: Insufficient tissue

TABLE G2
Individual Animal Respiratory System Tumor Pathology of Female Rats in the Lifetime Inhalation Study of Ozone: 0.5 ppm

Number of Days on Study	2	4	4	5	5	5	5	5	5	5	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	
	8	5	6	0	1	7	7	8	8	9	1	3	3	7	8	9	9	1	2	2	3	3	3	3	5	
	7	2	8	9	3	1	9	0	5	8	6	6	6	8	2	1	9	0	1	3	3	3	8	9	6	
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	1	2	1	4	3	4	2	5	5	0	5	0	2	2	3	1	4	2	4	3	1	3	2	1	0	
	8	0	6	2	7	0	2	3	0	2	4	1	3	8	5	0	7	1	5	3	4	0	7	1	5	
Respiratory System																										
Larynx	+	A	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma																		X								
Alveolar/bronchiolar carcinoma																										
Histiocytic sarcoma														X												
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										
Nose	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Number of Days on Study	7	7	7	7	7	7	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	
	5	6	7	7	7	9	0	0	1	1	1	2	2	3	3	4	4	4	5	7	7	7	7	7	7	
	6	7	5	5	8	3	3	3	0	7	7	4	7	0	8	2	2	9	9	4	4	4	4	4	4	
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Total
	3	1	0	3	3	1	0	2	4	3	5	0	2	4	3	0	1	4	2	0	0	1	4	5	5	Tissues/
	6	5	9	4	8	2	3	4	8	9	1	6	6	1	1	4	3	3	9	7	8	9	6	5	6	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Alveolar/bronchiolar carcinoma									X																	1
Histiocytic sarcoma																										1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung									X																	1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE G2
Individual Animal Respiratory System Tumor Pathology of Female Rats in the Lifetime Inhalation Study of Ozone: 1.0 ppm

Number of Days on Study	2	4	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	
	8	6	8	8	3	5	5	5	6	8	1	3	3	4	6	6	7	7	7	8	8	9	9	0	0	
	5	7	3	6	6	1	4	9	6	0	4	2	5	3	3	6	0	0	3	2	5	5	9	5	5	
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	5	5	4	1	2	5	0	1	5	0	3	4	2	1	0	0	1	1	4	0	2	4	2	2	3	
	0	5	8	4	9	2	1	7	6	4	2	9	6	3	8	9	8	9	3	2	1	4	4	7	0	
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Carcinoma, metastatic, thyroid gland																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma																					X					
Carcinoma, metastatic, thyroid gland																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Chondroma																										
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	8	8	8	8	8	8	8	8	8	8	8	8	8	
	1	1	2	3	3	4	5	5	6	7	7	9	0	1	1	3	4	6	7	7	7	7	7	7	7	
	3	4	1	3	3	4	3	8	1	5	5	3	0	1	7	1	8	1	4	4	4	4	4	4	4	
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Total
	5	3	4	1	2	2	3	1	4	3	5	3	3	0	4	1	2	0	0	0	1	2	3	4	4	Tissues/
	3	8	0	6	5	0	5	1	1	9	4	3	7	5	6	2	2	3	6	7	5	3	4	2	7	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland																									X	1
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Carcinoma, metastatic, thyroid gland																		X							X	2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Chondroma										X																1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE G3
Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Ozone

	0 ppm	0.5 ppm	1.0 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rate[a]	13/50 (26%)	15/49 (31%)	14/50 (28%)
Adjusted rate[b]	81.2%	67.5%	78.5%
Terminal rate[c]	4/6 (67%)	2/6 (33%)	4/7 (57%)
First incidence (days)	577	585	536
Life table test[d]	P=0.396N	P=0.332N	P=0.428N
Logistic regression test[d]	P=0.478N	P=0.489N	P=0.502N
Cochran-Armitage test[d]	P=0.456		
Fisher exact test[d]		P=0.387	P=0.500
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma			
Overall rate	13/50 (26%)	16/49 (33%)	14/50 (28%)
Adjusted rate	81.2%	75.6%	78.5%
Terminal rate	4/6 (67%)	3/6 (50%)	4/7 (57%)
First incidence (days)	577	585	536
Life table test	P=0.391N	P=0.409N	P=0.428N
Logistic regression test	P=0.469N	P=0.553N	P=0.502N
Cochran-Armitage test	P=0.456		
Fisher exact test		P=0.306	P=0.500
Clitoral Gland: Adenoma			
Overall rate	3/45 (7%)	3/48 (6%)	6/47 (13%)
Adjusted rate	13.8%	21.4%	41.8%
Terminal rate	0/6 (0%)	1/6 (17%)	1/7 (14%)
First incidence (days)	635	513	685
Life table test	P=0.254	P=0.526N	P=0.366
Logistic regression test	P=0.227	P=0.628N	P=0.344
Cochran-Armitage test	P=0.193		
Fisher exact test		P=0.630N	P=0.265
Clitoral Gland: Carcinoma			
Overall rate	0/45 (0%)	2/48 (4%)	3/47 (6%)
Adjusted rate	0.0%	8.0%	21.4%
Terminal rate	0/6 (0%)	0/6 (0%)	1/7 (14%)
First incidence (days)	–[e]	756	670
Life table test	P=0.109	P=0.407	P=0.167
Logistic regression test	P=0.099	P=0.300	P=0.157
Cochran-Armitage test	P=0.086		
Fisher exact test		P=0.264	P=0.129
Clitoral Gland: Adenoma or Carcinoma			
Overall rate	3/45 (7%)	5/48 (10%)	9/47 (19%)
Adjusted rate	13.8%	27.7%	55.6%
Terminal rate	0/6 (0%)	1/6 (17%)	2/7 (29%)
First incidence (days)	635	513	670
Life table test	P=0.083	P=0.576	P=0.140
Logistic regression test	P=0.062	P=0.426	P=0.112
Cochran-Armitage test	P=0.047		
Fisher exact test		P=0.394	P=0.070

TABLE G3
Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Mammary Gland: Carcinoma			
Overall rate	8/50 (16%)	5/50 (10%)	7/50 (14%)
Adjusted rate	43.4%	44.4%	45.7%
Terminal rate	2/6 (33%)	2/6 (33%)	2/7 (29%)
First incidence (days)	417	571	467
Life table test	P=0.334N	P=0.193N	P=0.385N
Logistic regression test	P=0.422N	P=0.270N	P=0.498N
Cochran-Armitage test	P=0.442N		
Fisher exact test		P=0.277N	P=0.500N
Mammary Gland: Adenoma or Carcinoma			
Overall rate	8/50 (16%)	5/50 (10%)	8/50 (16%)
Adjusted rate	43.4%	44.4%	47.2%
Terminal rate	2/6 (33%)	2/6 (33%)	2/7 (29%)
First incidence (days)	417	571	467
Life table test	P=0.444N	P=0.193N	P=0.485N
Logistic regression test	P=0.543N	P=0.270N	P=0.605
Cochran-Armitage test	P=0.557		
Fisher exact test		P=0.277N	P=0.607N
Mammary Gland: Fibroadenoma			
Overall rate	19/50 (38%)	28/50 (56%)	25/50 (50%)
Adjusted rate	93.1%	100.0%	79.3%
Terminal rate	5/6 (83%)	6/6 (100%)	2/7 (29%)
First incidence (days)	557	579	580
Life table test	P=0.450	P=0.528	P=0.481
Logistic regression test	P=0.234	P=0.288	P=0.250
Cochran-Armitage test	P=0.135		
Fisher exact test		P=0.054	P=0.157
Mammary Gland: Fibroadenoma or Adenoma			
Overall rate	19/50 (38%)	28/50 (56%)	25/50 (50%)
Adjusted rate	93.1%	100.0%	79.3%
Terminal rate	5/6 (83%)	6/6 (100%)	2/7 (29%)
First incidence (days)	557	579	580
Life table test	P=0.450	P=0.528	P=0.481
Logistic regression test	P=0.234	P=0.288	P=0.250
Cochran-Armitage test	P=0.135		
Fisher exact test		P=0.054	P=0.157
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma			
Overall rate	24/50 (48%)	29/50 (58%)	30/50 (60%)
Adjusted rate	93.9%	100.0%	89.9%
Terminal rate	5/6 (83%)	6/6 (100%)	4/7 (57%)
First incidence (days)	417	571	467
Life table test	P=0.478	P=0.337N	P=0.521
Logistic regression test	P=0.229	P=0.478	P=0.251
Cochran-Armitage test	P=0.134		
Fisher exact test		P=0.212	P=0.158

TABLE G3
Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	44/50 (88%)	40/49 (82%)	37/50 (74%)
Adjusted rate	100.0%	97.4%	100.0%
Terminal rate	6/6 (100%)	5/6 (83%)	7/7 (100%)
First incidence (days)	406	513	467
Life table test	P=0.047N	P=0.022N	P=0.061N
Logistic regression test	P=0.016N	P=0.074N	P=0.025N
Cochran-Armitage test	P=0.048N		
Fisher exact test		P=0.274N	P=0.062N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rate	44/50 (88%)	41/49 (84%)	37/50 (74%)
Adjusted rate	100.0%	97.6%	100.0%
Terminal rate	6/6 (100%)	5/6 (83%)	7/7 (100%)
First incidence (days)	406	513	467
Life table test	P=0.046N	P=0.028N	P=0.061N
Logistic regression test	P=0.014N	P=0.112N	P=0.025N
Cochran-Armitage test	P=0.046N		
Fisher exact test		P=0.371N	P=0.062N
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma			
Overall rate	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate	24.3%	0.0%	28.6%
Terminal rate	1/6 (17%)	0/6 (0%)	2/7 (29%)
First incidence (days)	691	–	874 (T)
Life table test	P=0.333N	P=0.080N	P=0.428N
Logistic regression test	P=0.315N	P=0.081N	P=0.401N
Cochran-Armitage test	P=0.390N		
Fisher exact test		P=0.121N	P=0.500N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	20.8%	16.7%	20.8%
Terminal rate	1/6 (17%)	1/6 (17%)	1/7 (14%)
First incidence (days)	722	874 (T)	643
Life table test	P=0.457	P=0.448N	P=0.570
Logistic regression test	P=0.456	P=0.353N	P=0.567
Cochran-Armitage test	P=0.399		
Fisher exact test		P=0.500N	P=0.500
Thyroid Gland (C-cell): Adenoma			
Overall rate	7/50 (14%)	6/49 (12%)	4/50 (8%)
Adjusted rate	56.9%	40.0%	26.5%
Terminal rate	2/6 (33%)	1/6 (17%)	1/7 (14%)
First incidence (days)	696	723	566
Life table test	P=0.127N	P=0.226N	P=0.173N
Logistic regression test	P=0.138N	P=0.234N	P=0.185N
Cochran-Armitage test	P=0.216N		
Fisher exact test		P=0.516N	P=0.262N

TABLE G3
Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Thyroid Gland (C-cell): Carcinoma			
Overall rate	4/50 (8%)	3/49 (6%)	4/50 (8%)
Adjusted rate	32.8%	16.0%	40.6%
Terminal rate	1/6 (17%)	0/6 (0%)	2/7 (29%)
First incidence (days)	678	767	744
Life table test	P=0.447N	P=0.237N	P=0.521N
Logistic regression test	P=0.478N	P=0.349N	P=0.528N
Cochran-Armitage test	P=0.576		
Fisher exact test		P=0.511N	P=0.643N
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rate	10/50 (20%)	9/49 (18%)	8/50 (16%)
Adjusted rate	65.3%	49.6%	59.3%
Terminal rate	2/6 (33%)	1/6 (17%)	3/7 (43%)
First incidence (days)	678	723	566
Life table test	P=0.193N	P=0.144N	P=0.242N
Logistic regression test	P=0.213N	P=0.130N	P=0.253N
Cochran-Armitage test	P=0.348N		
Fisher exact test		P=0.520N	P=0.398N
Uterus: Stromal Polyp			
Overall rate	6/50 (12%)	3/50 (6%)	6/50 (12%)
Adjusted rate	29.5%	23.8%	37.1%
Terminal rate	0/6 (0%)	1/6 (17%)	2/7 (29%)
First incidence (days)	556	616	486
Life table test	P=0.478N	P=0.105N	P=0.518N
Logistic regression test	P=0.548N	P=0.200N	P=0.602N
Cochran-Armitage test	P=0.566		
Fisher exact test		P=0.243N	P=0.620N
Uterus: Stromal Polyp or Stromal Sarcoma			
Overall rate	6/50 (12%)	3/50 (6%)	7/50 (14%)
Adjusted rate	29.5%	23.8%	42.8%
Terminal rate	0/6 (0%)	1/6 (17%)	2/7 (29%)
First incidence (days)	556	616	486
Life table test	P=0.532	P=0.105N	P=0.610N
Logistic regression test	P=0.462	P=0.200N	P=0.533
Cochran-Armitage test	P=0.436		
Fisher exact test		P=0.243N	P=0.500
All Organs: Mononuclear Cell Leukemia			
Overall rate	21/50 (42%)	22/50 (44%)	20/50 (40%)
Adjusted rate	74.7%	80.1%	85.6%
Terminal rate	2/6 (33%)	3/6 (50%)	5/7 (71%)
First incidence (days)	467	452	486
Life table test	P=0.256N	P=0.239N	P=0.284N
Logistic regression test	P=0.408N	P=0.566	P=0.427N
Cochran-Armitage test	P=0.460N		
Fisher exact test		P=0.500	P=0.500N

TABLE G3
Statistical Analysis of Primary Neoplasms in Female Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
All Organs: Benign Neoplasms			
Overall rate	47/50 (94%)	45/50 (90%)	48/50 (96%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	6/6 (100%)	6/6 (100%)	7/7 (100%)
First incidence (days)	406	513	467
Life table test	P=0.230N	P=0.035N	P=0.249N
Logistic regression test	P=0.525N	P=0.084N	P=0.705N
Cochran-Armitage test	P=0.421		
Fisher exact test		P=0.357N	P=0.500
All Organs: Malignant Neoplasms			
Overall rate	34/50 (68%)	31/50 (62%)	32/50 (64%)
Adjusted rate	93.1%	95.2%	96.0%
Terminal rate	4/6 (67%)	5/6 (83%)	6/7 (86%)
First incidence (days)	417	452	285
Life table test	P=0.168N	P=0.054N	P=0.187N
Logistic regression test	P=0.313N	P=0.238N	P=0.342N
Cochran-Armitage test	P=0.377N		
Fisher exact test		P=0.338N	P=0.417N
All Organs: Benign or Malignant Neoplasms			
Overall rate	49/50 (98%)	48/50 (96%)	50/50 (100%)
Adjusted rate	100.0%	100.0%	100.0%
Terminal rate	6/6 (100%)	6/6 (100%)	7/7 (100%)
First incidence (days)	406	452	285
Life table test	P=0.232N	P=0.047N	P=0.251N
Logistic regression test	P=0.605	P=0.242N	P=0.500
Cochran-Armitage test	P=0.360		
Fisher exact test		P=0.500N	P=0.500

(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, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.

[e] Not applicable; no neoplasms in animal group

TABLE G4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Lifetime Inhalation Study of Ozone[a]

	0 ppm	0.5 ppm	1.0 ppm
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	36	37	40
Natural death	8	7	3
Survivors			
Terminal sacrifice	6	6	7
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, colon	(49)	(50)	(50)
Parasite metazoan	3 (6%)	5 (10%)	4 (8%)
Intestine large, rectum	(48)	(50)	(47)
Parasite metazoan	2 (4%)	4 (8%)	5 (11%)
Intestine large, cecum	(48)	(49)	(50)
Inflammation, acute		1 (2%)	
Necrosis		1 (2%)	
Parasite metazoan	3 (6%)	3 (6%)	5 (10%)
Intestine small, jejunum	(47)	(47)	(49)
Hyperplasia, adenomatous	1 (2%)		
Liver	(50)	(50)	(50)
Angiectasis	1 (2%)	7 (14%)	3 (6%)
Basophilic focus	37 (74%)	39 (78%)	42 (84%)
Clear cell focus	1 (2%)	5 (10%)	5 (10%)
Degeneration, cystic			5 (10%)
Degeneration, fatty	18 (36%)	22 (44%)	11 (22%)
Eosinophilic focus	3 (6%)	3 (6%)	4 (8%)
Hematopoietic cell proliferation		1 (2%)	
Hepatodiaphragmatic nodule	3 (6%)	7 (14%)	12 (24%)
Inflammation, granulomatous		2 (4%)	
Mixed cell focus	11 (22%)	8 (16%)	9 (18%)
Necrosis	2 (4%)		3 (6%)
Thrombosis	1 (2%)		
Vacuolization cytoplasmic, focal	1 (2%)		
Bile duct, hyperplasia	15 (30%)	12 (24%)	10 (20%)
Centrilobular, necrosis	8 (16%)	5 (10%)	5 (10%)
Mesentery	(11)	(7)	(4)
Inflammation, chronic active	1 (9%)		
Artery, inflammation, chronic active		1 (14%)	
Artery, mineralization	1 (9%)	1 (14%)	
Fat, necrosis	6 (55%)	6 (86%)	4 (100%)
Oral mucosa	(2)	(2)	
Gingival, cyst	1 (50%)		
Pancreas	(49)	(49)	(50)
Atrophy	20 (41%)	14 (29%)	21 (42%)
Basophilic focus	1 (2%)	1 (2%)	
Hyperplasia	1 (2%)	1 (2%)	1 (2%)
Salivary glands	(50)	(50)	(50)
Basophilic focus			1 (2%)

TABLE G4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Alimentary System (continued)			
Stomach, forestomach	(49)	(50)	(50)
Hyperplasia, squamous	2 (4%)		3 (6%)
Mineralization	1 (2%)		
Necrosis	11 (22%)	9 (18%)	7 (14%)
Stomach, glandular	(49)	(50)	(50)
Inflammation, acute	1 (2%)		
Mineralization	3 (6%)	2 (4%)	4 (8%)
Necrosis	2 (4%)	2 (4%)	1 (2%)
Tongue		(1)	(1)
Hyperplasia		1 (100%)	
Tooth		(1)	
Developmental malformation		1 (100%)	
Cardiovascular System			
Blood vessel	(1)	(1)	(1)
Aorta, mineralization	1 (100%)	1 (100%)	1 (100%)
Heart	(50)	(50)	(50)
Cardiomyopathy	30 (60%)	35 (70%)	35 (70%)
Artery, mineralization	1 (2%)	1 (2%)	1 (2%)
Atrium, thrombosis	1 (2%)	4 (8%)	1 (2%)
Endocrine System			
Adrenal cortex	(50)	(49)	(50)
Atrophy	1 (2%)	3 (6%)	1 (2%)
Hyperplasia	19 (38%)	25 (51%)	22 (44%)
Hypertrophy	6 (12%)	5 (10%)	6 (12%)
Necrosis	1 (2%)	1 (2%)	1 (2%)
Thrombosis	1 (2%)	2 (4%)	
Vacuolization cytoplasmic	9 (18%)	7 (14%)	8 (16%)
Adrenal medulla	(50)	(49)	(50)
Hyperplasia	12 (24%)	18 (37%)	13 (26%)
Islets, pancreatic	(49)	(49)	(50)
Hyperplasia	2 (4%)	1 (2%)	1 (2%)
Parathyroid gland	(49)	(48)	(44)
Hyperplasia	4 (8%)	2 (4%)	2 (5%)
Pituitary gland	(50)	(49)	(50)
Cyst	1 (2%)		2 (4%)
Pars distalis, hyperplasia	3 (6%)	5 (10%)	10 (20%)
Thyroid gland	(50)	(49)	(50)
C-cell, hyperplasia	37 (74%)	38 (78%)	30 (60%)
Follicular cell, hyperplasia	1 (2%)		1 (2%)
General Body System			
None			
Genital System			
Clitoral gland	(45)	(48)	(47)
Hyperplasia			1 (2%)
Inflammation, chronic active	4 (9%)	3 (6%)	2 (4%)

TABLE G4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Genital System (continued)			
Ovary	(50)	(50)	(50)
Cyst	3 (6%)	3 (6%)	
Hyperplasia			1 (2%)
Uterus	(50)	(50)	(50)
Cyst	1 (2%)		
Thrombosis		1 (2%)	
Endometrium, hyperplasia		1 (2%)	
Hematopoietic System			
Bone marrow	(50)	(49)	(50)
Atrophy	1 (2%)		1 (2%)
Inflammation, granulomatous		1 (2%)	1 (2%)
Thrombosis		1 (2%)	
Lymph node	(10)	(12)	(9)
Pancreatic, inflammation, granulomatous		1 (8%)	
Renal, hemorrhage	1 (10%)	1 (8%)	
Renal, infiltration cellular, plasma cell			1 (11%)
Renal, inflammation, granulomatous	1 (10%)		
Lymph node, mandibular	(47)	(44)	(46)
Infiltration cellular, plasma cell	4 (9%)	1 (2%)	1 (2%)
Spleen	(50)	(50)	(50)
Depletion cellular		1 (2%)	
Fibrosis	6 (12%)	6 (12%)	3 (6%)
Hematopoietic cell proliferation	1 (2%)	2 (4%)	2 (4%)
Hemorrhage		2 (4%)	
Necrosis		1 (2%)	
Thrombosis	1 (2%)		
Integumentary System			
Mammary gland	(50)	(50)	(50)
Galactocele	1 (2%)		3 (6%)
Skin	(49)	(50)	(50)
Cyst	1 (2%)		
Hyperkeratosis	1 (2%)		
Inflammation, chronic active	6 (12%)	5 (10%)	2 (4%)
Musculoskeletal System			
Bone	(50)	(50)	(50)
Fibrous osteodystrophy	1 (2%)	3 (6%)	2 (4%)
Fracture	1 (2%)		
Hyperostosis	2 (4%)	6 (12%)	2 (4%)
Nervous System			
Brain	(50)	(50)	(50)
Gliosis			1 (2%)
Hemorrhage	2 (4%)	1 (2%)	
Mineralization	1 (2%)		1 (2%)
Necrosis	1 (2%)		
Thrombosis	1 (2%)		

TABLE G4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Respiratory System			
Larynx	(49)	(47)	(50)
Inflammation, chronic active	1 (2%)		
Epiglottis, metaplasia, squamous	2 (4%)	16 (34%)	48 (96%)
Lung	(50)	(50)	(50)
Congestion, chronic		1 (2%)	
Hemorrhage		1 (2%)	
Inflammation, chronic active	1 (2%)	1 (2%)	2 (4%)
Mineralization	1 (2%)	1 (2%)	1 (2%)
Alveolar epithelium, hyperplasia	4 (8%)	5 (10%)	2 (4%)
Alveolar epithelium, metaplasia		44 (88%)	50 (100%)
Alveolus, infiltration cellular, histiocyte		38 (76%)	49 (98%)
Artery, infiltration cellular, histiocyte	1 (2%)		
Artery, mediastinum, mineralization	1 (2%)	1 (2%)	1 (2%)
Artery, perivascular, inflammation, chronic	1 (2%)		
Interstitium, fibrosis		41 (82%)	50 (100%)
Nose	(50)	(49)	(50)
Inflammation, suppurative	6 (12%)	7 (14%)	10 (20%)
Thrombosis	8 (16%)	7 (14%)	3 (6%)
Goblet cell, lateral wall, hyperplasia		47 (96%)	50 (100%)
Lateral wall, hyperplasia	4 (8%)	49 (100%)	50 (100%)
Lateral wall, metaplasia, squamous	5 (10%)	25 (51%)	35 (70%)
Olfactory epithelium, degeneration, hyaline	48 (96%)	48 (98%)	50 (100%)
Olfactory epithelium, metaplasia		3 (6%)	4 (8%)
Trachea	(50)	(50)	(50)
Mineralization			1 (2%)
Special Senses System			
Eye		(1)	(2)
Cataract			1 (50%)
Degeneration			2 (100%)
Cornea, inflammation, chronic active		1 (100%)	
Cornea, mineralization		1 (100%)	
Urinary System			
Kidney	(50)	(50)	(50)
Cyst	1 (2%)		
Infarct	1 (2%)		
Mineralization	1 (2%)	1 (2%)	1 (2%)
Nephropathy	49 (98%)	47 (94%)	49 (98%)
Thrombosis			1 (2%)
Pelvis, dilatation	1 (2%)	1 (2%)	
Pelvis, inflammation, acute			1 (2%)
Renal tubule, hyperplasia	1 (2%)	1 (2%)	1 (2%)
Renal tubule, necrosis	1 (2%)	1 (2%)	

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

APPENDIX H
SUMMARY OF LESIONS IN MALE MICE IN THE LIFETIME INHALATION STUDY OF OZONE

TABLE H1 Summary of the Incidence of Neoplasms in Male Mice in the Lifetime Inhalation Study of Ozone 224

TABLE H2 Individual Animal Respiratory System Tumor Pathology of Male Mice in the Lifetime Inhalation Study of Ozone 228

TABLE H3 Statistical Analysis of Primary Neoplasms in Male Mice in the Lifetime Inhalation Study of Ozone 231

TABLE H4 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Lifetime Inhalation Study of Ozone 235

TABLE H1
Summary of the Incidence of Neoplasms in Male Mice in the Lifetime Inhalation Study of Ozone[a]

	0 ppm	0.5 ppm	1 ppm
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	26	30	23
Natural deaths	10	9	15
Survivors			
Terminal sacrifice	14	11	12
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, cecum	(44)	(45)	(40)
Carcinoma			1 (3%)
Intestine small, duodenum	(44)	(45)	(38)
Intestine small, jejunum	(43)	(44)	(41)
Carcinoma	1 (2%)	1 (2%)	
Sarcoma, metastatic, uncertain primary site			1 (2%)
Intestine small, ileum	(44)	(45)	(40)
Liver	(49)	(50)	(50)
Cholangiocarcinoma	1 (2%)		
Hemangiosarcoma		1 (2%)	
Hepatocellular carcinoma	7 (14%)	14 (28%)	14 (28%)
Hepatocellular carcinoma, multiple	13 (27%)	3 (6%)	7 (14%)
Hepatocellular adenoma	6 (12%)	12 (24%)	11 (22%)
Hepatocellular adenoma, multiple	7 (14%)	6 (12%)	1 (2%)
Hepatocholangiocarcinoma			2 (4%)
Histiocytic sarcoma	1 (2%)	1 (2%)	
Sarcoma, metastatic, seminal vesicle	1 (2%)		
Sarcoma, metastatic, uncertain primary site			1 (2%)
Squamous cell carcinoma, metastatic, uncertain primary site	1 (2%)		
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (2%)		
Mesentery	(3)	(3)	(4)
Hemangioma		1 (33%)	
Hemangiosarcoma		1 (33%)	
Sarcoma, metastatic, seminal vesicle	1 (33%)		
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (33%)		
Oral mucosa		(1)	
Pharyngeal, squamous cell carcinoma		1 (100%)	
Pancreas	(49)	(49)	(49)
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (2%)		
Salivary glands	(49)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		
Carcinoma	1 (2%)		
Stomach, forestomach	(49)	(49)	(50)
Sarcoma, metastatic, seminal vesicle	1 (2%)		
Squamous cell carcinoma	1 (2%)		
Squamous cell papilloma	2 (4%)		1 (2%)

TABLE H1
Summary of the Incidence of Neoplasms in Male Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1 ppm
Alimentary System (continued)			
Stomach, glandular	(49)	(47)	(48)
Sarcoma, metastatic, seminal vesicle	1 (2%)		
Tooth			(1)
Odontoma			1 (100%)
Cardiovascular System			
Heart	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		
Endocrine System			
Adrenal cortex	(47)	(49)	(49)
Adenoma	2 (4%)	1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver			1 (2%)
Sarcoma, metastatic, seminal vesicle	1 (2%)		
Adrenal medulla	(48)	(48)	(49)
Pheochromocytoma benign	1 (2%)		
Sarcoma, metastatic, seminal vesicle	1 (2%)		
Islets, pancreatic	(49)	(49)	(49)
Adenoma		1 (2%)	
Pituitary gland	(47)	(49)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		
Pars distalis, adenoma		1 (2%)	
Pars intermedia, adenoma	2 (4%)		
Thyroid gland	(49)	(48)	(50)
Follicular cell, adenoma	1 (2%)		
Follicular cell, carcinoma	1 (2%)		
General Body System			
None			
Genital System			
Epididymis	(49)	(49)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	1 (2%)
Sarcoma		1 (2%)	
Preputial gland	(49)	(49)	(49)
Adenoma			1 (2%)
Histiocytic sarcoma	1 (2%)		
Sarcoma		1 (2%)	
Prostate	(48)	(47)	(47)
Seminal vesicle	(48)	(49)	(49)
Sarcoma	1 (2%)		
Testes	(50)	(49)	(50)
Histiocytic sarcoma	1 (2%)		
Interstitial cell, adenoma	2 (4%)	1 (2%)	2 (4%)

TABLE H1
Summary of the Incidence of Neoplasms in Male Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1 ppm
Hematopoietic System			
Bone marrow	(49)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		
Hemangiosarcoma	1 (2%)	2 (4%)	2 (4%)
Histiocytic sarcoma	1 (2%)		
Femoral, mast cell tumor NOS			1 (2%)
Lymph node	(2)	(3)	(7)
Lymph node, bronchial	(26)	(27)	(32)
Sarcoma, metastatic, uncertain primary site			1 (3%)
Lymph node, mandibular	(34)	(31)	(32)
Lymph node, mesenteric	(48)	(46)	(44)
Histiocytic sarcoma		1 (2%)	
Sarcoma, metastatic, uncertain primary site			1 (2%)
Lymph node, mediastinal	(37)	(39)	(43)
Carcinoma, metastatic, harderian gland			1 (2%)
Spleen	(49)	(49)	(50)
Hemangiosarcoma	1 (2%)	3 (6%)	4 (8%)
Mast cell tumor NOS			1 (2%)
Thymus	(29)	(26)	(25)
Integumentary System			
Skin	(49)	(50)	(50)
Hemangiosarcoma			1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)	2 (4%)	
Subcutaneous tissue, sarcoma		2 (4%)	
Musculoskeletal System			
Bone	(50)	(50)	(50)
Osteosarcoma			1 (2%)
Skeletal muscle	(2)		(1)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (50%)		
Sarcoma, metastatic, seminal vesicle	1 (50%)		
Sarcoma, metastatic, uncertain primary site			1 (100%)
Nervous System			
Brain	(49)	(49)	(50)
Choristoma	1 (2%)		
Respiratory System			
Lung	(49)	(49)	(50)
Alveolar/bronchiolar adenoma	7 (14%)	8 (16%)	8 (16%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)		1 (2%)
Alveolar/bronchiolar carcinoma	6 (12%)	10 (20%)	14 (28%)
Alveolar/bronchiolar carcinoma, multiple	2 (4%)	5 (10%)	4 (8%)
Carcinoma, metastatic, harderian gland		2 (4%)	1 (2%)
Carcinoma, metastatic, salivary glands	1 (2%)		
Hepatocellular carcinoma, metastatic, liver	6 (12%)	4 (8%)	7 (14%)
Hepatocholangiocarcinoma, metastatic, liver			2 (4%)

TABLE H1
Summary of the Incidence of Neoplasms in Male Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1 ppm
Respiratory System (continued)			
Lung (continued)	(49)	(49)	(50)
Histiocytic sarcoma		1 (2%)	
Osteosarcoma, metastatic, bone			1 (2%)
Sarcoma, metastatic, uncertain primary site			1 (2%)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)	1 (2%)	
Mediastinum, hemangioma		1 (2%)	
Mediastinum, hepatocholangiocarcinoma, metastatic, liver			1 (2%)
Nose	(49)	(48)	(49)
Histiocytic sarcoma	1 (2%)		
Special Senses System			
Harderian gland	(7)	(8)	(5)
Adenoma	6 (86%)	5 (63%)	4 (80%)
Carcinoma		2 (25%)	1 (20%)
Urinary System			
Kidney	(49)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		
Histiocytic sarcoma	1 (2%)	1 (2%)	
Urinary bladder	(47)	(49)	(48)
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	1 (2%)
Lymphoma malignant	2 (4%)	3 (6%)	3 (6%)
Neoplasm Summary			
Total animals with primary neoplasms[c]	43	50	42
Total primary neoplasms	78	90	88
Total animals with benign neoplasms	28	32	23
Total benign neoplasms	37	37	31
Total animals with malignant neoplasms	30	41	40
Total malignant neoplasms	40	53	55
Total animals with metastatic neoplasms	11	7	12
Total metastatic neoplasms	25	7	20
Total animals with malignant neoplasms uncertain primary site	1		1
Total animals with uncertain neoplasms - benign or malignant	1		1
Total uncertain neoplasms	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 H2
Individual Animal Respiratory System Tumor Pathology of Male Mice in the Lifetime Inhalation Study of Ozone: 0 ppm

Number of Days on Study	0	3	3	3	3	4	4	5	5	6	6	6	6	7	7	7	7	7	7	7	7	8	8	8	
	9	2	8	9	9	5	8	2	7	0	6	9	9	0	0	2	2	4	6	7	8	9	0	0	
	5	8	7	1	2	4	2	7	8	5	5	3	4	1	7	1	2	9	7	8	1	1	5	7	7
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	1	2	2	1	4	0	1	2	0	3	4	2	2	0	4	3	4	2	1	0	0	4	1	0	0
	8	9	7	1	8	6	4	6	8	7	6	3	4	5	2	8	3	2	3	4	2	0	0	7	9
Respiratory System																									
Larynx	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X					X							X									
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma																									
Alveolar/bronchiolar carcinoma, multiple																							X		
Carcinoma, metastatic, salivary glands					X																				
Hepatocellular carcinoma, metastatic, liver		X	X					X									X				X	X			
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																							X		
Nose	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									X
Trachea	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	8	8	8	8	8	8	8	8	8	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	
	0	1	1	4	4	4	6	8	8	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	7	0	7	0	5	7	9	2	3	1	3	1	1	1	1	1	1	1	1	2	2	2	2	2	2	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	Total
	1	3	3	3	2	2	4	3	0	1	5	1	2	3	3	3	4	4	4	0	1	1	2	3	4	Tissues/
	2	9	4	3	0	5	7	1	3	6	0	5	1	0	2	5	1	5	9	1	7	9	8	6	4	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma			X				X						X			X										7
Alveolar/bronchiolar adenoma, multiple																							X			1
Alveolar/bronchiolar carcinoma						X		X			X				X		X				X					6
Alveolar/bronchiolar carcinoma, multiple																			X							2
Carcinoma, metastatic, salivary glands																										1
Hepatocellular carcinoma, metastatic, liver																										6
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

+: Tissue examined microscopically X: Lesion present A: Autolysis precludes examination

TABLE H2
Individual Animal Respiratory System Tumor Pathology of Male Mice in the Lifetime Inhalation Study of Ozone: 0.5 ppm

Number of Days on Study	3	4	4	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7
	8	4	7	6	1	1	3	4	5	7	7	9	9	0	0	1	2	2	2	3	3	7	8	9	9
	6	6	1	8	0	6	2	9	1	8	8	3	3	7	9	6	1	1	2	5	5	3	7	1	8
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	3	0	1	0	5	1	3	0	2	2	0	0	3	1	2	2	4	2	3	4	1	4	2	0
	7	7	9	5	6	0	8	0	8	4	8	4	7	8	7	6	0	9	2	2	1	9	8	9	2
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X				X			X				X	X		
Alveolar/bronchiolar carcinoma																	X			X	X				
Alveolar/bronchiolar carcinoma, multiple												X				X									
Carcinoma, metastatic, harderian gland																									
Hepatocellular carcinoma, metastatic, liver		X	X		X									X											
Histiocytic sarcoma															X										
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																									
Mediastinum, hemangioma																				X					
Nose	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	7	8	8	8	8	8	8	8	8	8	8	8	8	9	9	9	9	9	9	9	9	9	9	9	9	
	9	0	0	1	2	2	3	4	4	4	6	7	8	0	1	1	1	1	1	1	1	1	1	1	1	
	9	4	5	2	3	6	8	5	7	7	9	5	0	1	1	1	1	1	1	2	2	2	2	2	2	
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Total
	1	1	4	4	1	3	4	3	3	3	0	4	4	2	0	1	1	3	4	0	1	2	2	3	4	Tissues/
	6	4	2	0	1	9	7	4	1	5	3	4	3	3	5	0	2	3	5	1	3	1	5	6	6	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma		X																	X		X					8
Alveolar/bronchiolar carcinoma										X	X				X			X	X			X			X	10
Alveolar/bronchiolar carcinoma, multiple			X	X									X													5
Carcinoma, metastatic, harderian gland						X										X										2
Hepatocellular carcinoma, metastatic, liver																										4
Histiocytic sarcoma																										1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung											X															1
Mediastinum, hemangioma																										1
Nose	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE H2
Individual Animal Respiratory System Tumor Pathology of Male Mice in the Lifetime Inhalation Study of Ozone: 1.0 ppm

Number of Days on Study	2	4	4	4	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7
	9	0	3	6	5	6	7	8	0	1	2	2	2	6	7	7	7	7	8	1	3	3	4	4	7
	7	8	5	9	0	1	2	1	9	6	0	3	8	5	0	7	7	8	5	9	0	2	1	9	8
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	2	4	3	0	3	2	2	2	4	2	1	2	1	0	2	0	3	4	3	5	4	0	3	0	1
	7	8	6	8	2	3	4	0	5	8	4	9	0	7	1	3	9	6	4	0	3	9	8	4	5
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X	X													
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma									X				X				X							X	
Alveolar/bronchiolar carcinoma, multiple														X											
Carcinoma, metastatic, harderian gland																									
Hepatocellular carcinoma, metastatic, liver									X							X		X	X						
Hepatocholangiocarcinoma, metastatic, liver																					X				
Osteosarcoma, metastatic, bone																							X		
Sarcoma, metastatic, uncertain primary site										X															
Mediastinum, hepatocholangiocarcinoma, metastatic, liver																					X				
Nose	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Number of Days on Study	7	7	8	8	8	8	8	8	8	8	8	8	8	9	9	9	9	9	9	9	9	9	9	9	9	
	8	9	0	0	0	1	1	2	3	3	7	7	7	1	1	1	1	1	1	1	1	1	1	1	1	
	6	1	7	7	8	2	9	8	2	8	4	5	7	1	1	1	1	2	2	2	2	2	2	2	2	
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Total
	1	2	1	1	3	3	0	4	2	1	2	4	4	0	1	3	4	0	0	1	1	3	3	4	4	Tissues/
	7	5	3	9	3	5	6	4	6	1	2	7	1	1	8	7	2	2	5	2	6	0	1	0	9	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X											X					X						X	X	X	8
Alveolar/bronchiolar adenoma, multiple														X												1
Alveolar/bronchiolar carcinoma	X			X				X				X		X		X	X					X	X	X		14
Alveolar/bronchiolar carcinoma, multiple						X			X		X															4
Carcinoma, metastatic, harderian gland							X																			1
Hepatocellular carcinoma, metastatic, liver									X				X												X	7
Hepatocholangiocarcinoma, metastatic, liver										X																2
Osteosarcoma, metastatic, bone																										1
Sarcoma, metastatic, uncertain primary site																										1
Mediastinum, hepatocholangiocarcinoma, metastatic, liver																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE H3
Statistical Analysis of Primary Neoplasms in Male Mice in the Lifetime Inhalation Study of Ozone

	0 ppm	0.5 ppm	1.0 ppm
Harderian Gland: Adenoma			
Overall rate[a]	6/50 (12%)	5/50 (10%)	4/50 (8%)
Adjusted rate[b]	24.9%	18.3%	25.4%
Terminal rate[c]	2/14 (14%)	0/11 (0%)	1/12 (8%)
First incidence (days)	482	616	828
Life table test[d]	P=0.419N	P=0.575N	P=0.475N
Logistic regression test[d]	P=0.326N	P=0.497N	P=0.410N
Cochran-Armitage test[d]	P=0.309N		
Fisher exact test[d]		P=0.500N	P=0.370N
Harderian Gland: Adenoma or Carcinoma			
Overall rate	6/50 (12%)	7/50 (14%)	5/50 (10%)
Adjusted rate	24.9%	29.4%	29.3%
Terminal rate	2/14 (14%)	1/11 (9%)	1/12 (8%)
First incidence (days)	482	616	819
Life table test	P=0.555	P=0.412	P=0.607N
Logistic regression test	P=0.471N	P=0.508	P=0.547N
Cochran-Armitage test	P=0.439N		
Fisher exact test		P=0.500	P=0.500N
Liver: Hepatocellular Adenoma			
Overall rate	13/49 (27%)	18/50 (36%)	12/50 (24%)
Adjusted rate	64.8%	72.2%	55.2%
Terminal rate	8/14 (57%)	6/11 (55%)	4/12 (33%)
First incidence (days)	605	386	469
Life table test	P=0.438	P=0.095	P=0.515
Logistic regression test	P=0.530N	P=0.196	P=0.578
Cochran-Armitage test	P=0.431N		
Fisher exact test		P=0.212	P=0.477N
Liver: Hepatocellular Carcinoma			
Overall rate	20/49 (41%)	17/50 (34%)	21/50 (42%)
Adjusted rate	57.4%	52.3%	57.1%
Terminal rate	2/14 (14%)	2/11 (18%)	2/12 (17%)
First incidence (days)	328	446	297
Life table test	P=0.302	P=0.480N	P=0.335
Logistic regression test	P=0.528N	P=0.320N	P=0.565N
Cochran-Armitage test	P=0.491		
Fisher exact test		P=0.311N	P=0.534
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	31/49 (63%)	34/50 (68%)	31/50 (62%)
Adjusted rate	87.0%	87.1%	81.3%
Terminal rate	10/14 (71%)	7/11 (64%)	6/12 (50%)
First incidence (days)	328	386	297
Life table test	P=0.284	P=0.217	P=0.325
Logistic regression test	P=0.484N	P=0.389	P=0.538N
Cochran-Armitage test	P=0.488N		
Fisher exact test		P=0.388	P=0.531N

TABLE H3
Statistical Analysis of Primary Neoplasms in Male Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	8/49 (16%)	8/49 (16%)	9/50 (18%)
Adjusted rate	33.9%	32.8%	50.6%
Terminal rate	3/14 (21%)	2/11 (18%)	5/12 (42%)
First incidence (days)	391	678	620
Life table test	P=0.332	P=0.518	P=0.389
Logistic regression test	P=0.427	P=0.606N	P=0.473
Cochran-Armitage test	P=0.465		
Fisher exact test		P=0.607N	P=0.518
Lung: Alveolar/bronchiolar Carcinoma			
Overall rate	8/49 (16%)	15/49 (31%)	18/50 (36%)
Adjusted rate	42.3%	65.3%	70.9%
Terminal rate	4/14 (29%)	5/11 (45%)	6/12 (50%)
First incidence (days)	805	693	609
Life table test	P=0.007	P=0.033	P=0.009
Logistic regression test	P=0.005	P=0.050	P=0.007
Cochran-Armitage test	P=0.019		
Fisher exact test		P=0.076	P=0.022
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	16/49 (33%)	22/49 (45%)	21/50 (42%)
Adjusted rate	66.0%	76.3%	77.0%
Terminal rate	7/14 (50%)	6/11 (55%)	7/12 (58%)
First incidence (days)	391	678	609
Life table test	P=0.086	P=0.078	P=0.107
Logistic regression test	P=0.127	P=0.140	P=0.149
Cochran-Armitage test	P=0.200		
Fisher exact test		P=0.150	P=0.226
Spleen: Hemangiosarcoma			
Overall rate	1/49 (2%)	3/49 (6%)	4/50 (8%)
Adjusted rate	3.4%	14.5%	18.8%
Terminal rate	0/14 (0%)	0/11 (0%)	1/12 (8%)
First incidence (days)	791	787	677
Life table test	P=0.096	P=0.262	P=0.144
Logistic regression test	P=0.121	P=0.301	P=0.170
Cochran-Armitage test	P=0.138		
Fisher exact test		P=0.309	P=0.187
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma			
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	13.8%	0.0%	7.1%
Terminal rate	1/14 (7%)	0/11 (0%)	0/12 (0%)
First incidence (days)	701	–[e]	875
Life table test	P=0.237N	P=0.151N	P=0.396N
Logistic regression test	P=0.194N	P=0.120N	P=0.336N
Cochran-Armitage test	P=0.176N		
Fisher exact test		P=0.121N	P=0.309N

TABLE H3
Statistical Analysis of Primary Neoplasms in Male Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
All Organs: Hemangiosarcoma			
Overall rate	2/50 (4%)	5/50 (10%)	5/50 (10%)
Adjusted rate	10.3%	18.4%	23.3%
Terminal rate	1/14 (7%)	0/11 (0%)	1/12 (8%)
First incidence (days)	791	568	677
Life table test	P=0.130	P=0.190	P=0.166
Logistic regression test	P=0.169	P=0.219	P=0.189
Cochran-Armitage test	P=0.178		
Fisher exact test		P=0.218	P=0.218
All Organs: Hemangioma or Hemangiosarcoma			
Overall rate	2/50 (4%)	7/50 (14%)	5/50 (10%)
Adjusted rate	10.3%	28.2%	23.3%
Terminal rate	1/14 (7%)	1/11 (9%)	1/12 (8%)
First incidence (days)	791	568	677
Life table test	P=0.140	P=0.067	P=0.166
Logistic regression test	P=0.181	P=0.083	P=0.189
Cochran-Armitage test	P=0.195		
Fisher exact test		P=0.080	P=0.218
All Organs: Malignant Lymphoma (Histiocytic or Lymphocytic)			
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)
Adjusted rate	9.4%	14.0%	11.1%
Terminal rate	1/14 (7%)	1/11 (9%)	0/12 (0%)
First incidence (days)	578	386	623
Life table test	P=0.369	P=0.463	P=0.457
Logistic regression test	P=0.413	P=0.475	P=0.501
Cochran-Armitage test	P=0.412		
Fisher exact test		P=0.500	P=0.500
All Organs: Malignant Lymphoma or Histiocytic Sarcoma			
Overall rate	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted rate	12.7%	16.4%	18.5%
Terminal rate	1/14 (7%)	1/11 (9%)	1/12 (8%)
First incidence (days)	578	386	623
Life table test	P=0.367	P=0.455	P=0.440
Logistic regression test	P=0.425	P=0.481	P=0.492
Cochran-Armitage test	P=0.424		
Fisher exact test		P=0.500	P=0.500
All Organs: Benign Neoplasms			
Overall rate	28/50 (56%)	32/50 (64%)	23/50 (46%)
Adjusted rate	88.8%	86.8%	87.2%
Terminal rate	11/14 (79%)	7/11 (64%)	9/12 (75%)
First incidence (days)	391	386	469
Life table test	P=0.500N	P=0.157	P=0.484N
Logistic regression test	P=0.214N	P=0.311	P=0.268N
Cochran-Armitage test	P=0.183N		
Fisher exact test		P=0.270	P=0.212N

TABLE H3
Statistical Analysis of Primary Neoplasms in Male Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
All Organs: Malignant Neoplasms			
Overall rate	30/50 (60%)	41/50 (82%)	40/50 (80%)
Adjusted rate	74.8%	92.6%	88.4%
Terminal rate	5/14 (36%)	8/11 (73%)	7/12 (58%)
First incidence (days)	328	386	297
Life table test	P=0.033	P=0.041	P=0.043
Logistic regression test	P=0.015	P=0.013	P=0.025
Cochran-Armitage test	P=0.015		
Fisher exact test		P=0.013	P=0.024
All Organs: Benign or Malignant Neoplasms			
Overall rate	43/50 (86%)	50/50 (100%)	42/50 (84%)
Adjusted rate	93.2%	100.0%	93.0%
Terminal rate	11/14 (79%)	11/11 (100%)	9/12 (75%)
First incidence (days)	328	386	297
Life table test	P=0.276	P=0.099	P=0.331
Logistic regression test	P=0.287N	P=0.010	P=0.503N
Cochran-Armitage test	P=0.434N		
Fisher exact test		P=0.006	P=0.500N

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and spleen; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE H4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Lifetime Inhalation Study of Ozone[a]

	0 ppm	0.5 ppm	1.0 ppm
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	26	30	23
Natural deaths	10	9	15
Survivors			
Terminal sacrifice	14	11	12
Animals examined microscopically	50	50	50
Alimentary System			
Gallbladder	(43)	(44)	(40)
Inflammation, suppurative		1 (2%)	2 (5%)
Mineralization			1 (3%)
Epithelium, hyperplasia		1 (2%)	
Intestine small, duodenum	(44)	(45)	(38)
Necrosis	1 (2%)		
Intestine small, jejunum	(43)	(44)	(41)
Peyer's patch, hyperplasia			2 (5%)
Liver	(49)	(50)	(50)
Angiectasis			1 (2%)
Basophilic focus	2 (4%)	1 (2%)	2 (4%)
Clear cell focus	1 (2%)		
Degeneration, fatty	2 (4%)	1 (2%)	2 (4%)
Eosinophilic focus	10 (20%)	9 (18%)	3 (6%)
Hematopoietic cell proliferation	2 (4%)	1 (2%)	1 (2%)
Hepatodiaphragmatic nodule	1 (2%)		1 (2%)
Infiltration cellular, mast cell			1 (2%)
Inflammation, chronic	2 (4%)	1 (2%)	1 (2%)
Necrosis	3 (6%)	8 (16%)	3 (6%)
Bile duct, cyst	1 (2%)	1 (2%)	2 (4%)
Centrilobular, necrosis		1 (2%)	1 (2%)
Mesentery	(3)	(3)	(4)
Artery, inflammation, chronic active			1 (25%)
Fat, necrosis	1 (33%)	1 (33%)	2 (50%)
Pancreas	(49)	(49)	(49)
Atrophy	3 (6%)	3 (6%)	1 (2%)
Basophilic focus	1 (2%)		
Cytoplasmic alteration			1 (2%)
Vacuolization cytoplasmic	1 (2%)		
Duct, cyst		1 (2%)	
Stomach, forestomach	(49)	(49)	(50)
Angiectasis			1 (2%)
Infiltration cellular, mast cell			1 (2%)
Inflammation, suppurative		2 (4%)	
Necrosis			3 (6%)
Epithelium, hyperplasia	1 (2%)	4 (8%)	3 (6%)
Stomach, glandular	(49)	(47)	(48)
Inflammation, acute	1 (2%)	1 (2%)	
Mineralization		1 (2%)	
Necrosis	2 (4%)	3 (6%)	1 (2%)
Epithelium, hyperplasia	1 (2%)		1 (2%)

TABLE H4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Cardiovascular System			
Blood vessel			(1)
Mineralization			1 (100%)
Heart	(50)	(50)	(50)
Cardiomyopathy	40 (80%)	40 (80%)	40 (80%)
Inflammation, suppurative	2 (4%)		
Mineralization		1 (2%)	
Necrosis	1 (2%)		1 (2%)
Artery, inflammation, chronic active			1 (2%)
Atrium, thrombosis	2 (4%)	1 (2%)	2 (4%)
Endocrine System			
Adrenal cortex	(47)	(49)	(49)
Hyperplasia	9 (19%)	13 (27%)	8 (16%)
Hypertrophy	20 (43%)	16 (33%)	13 (27%)
Capsule, hyperplasia	9 (19%)	12 (24%)	7 (14%)
Adrenal medulla	(48)	(48)	(49)
Hyperplasia	2 (4%)	2 (4%)	
Thrombosis		1 (2%)	
Islets, pancreatic	(49)	(49)	(49)
Hyperplasia	4 (8%)	1 (2%)	2 (4%)
Pituitary gland	(47)	(49)	(48)
Pars distalis, hyperplasia	1 (2%)	4 (8%)	3 (6%)
Pars intermedia, hyperplasia	1 (2%)		2 (4%)
Thyroid gland	(49)	(48)	(50)
Follicular cell, hyperplasia	18 (37%)	20 (42%)	32 (64%)
General Body System			
None			
Genital System			
Epididymis	(49)	(49)	(50)
Atrophy		1 (2%)	
Granuloma sperm	1 (2%)	1 (2%)	
Inflammation	2 (4%)		1 (2%)
Inflammation, chronic		1 (2%)	
Penis	(4)	(2)	(7)
Inflammation, suppurative	3 (75%)	2 (100%)	5 (71%)
Preputial gland	(49)	(49)	(49)
Cyst	11 (22%)	11 (22%)	8 (16%)
Hyperplasia			1 (2%)
Inflammation, chronic active	15 (31%)	13 (27%)	9 (18%)
Prostate	(48)	(47)	(47)
Hyperplasia	1 (2%)		
Inflammation, suppurative	3 (6%)	5 (11%)	3 (6%)
Artery, inflammation, chronic active			1 (2%)
Seminal vesicle	(48)	(49)	(49)
Hyperplasia			1 (2%)
Inflammation, suppurative	1 (2%)		1 (2%)

TABLE H4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Genital System (continued)			
Testes	(50)	(49)	(50)
Atrophy	11 (22%)	10 (20%)	5 (10%)
Mineralization		1 (2%)	1 (2%)
Interstitial cell, hyperplasia	2 (4%)	1 (2%)	
Hematopoietic System			
Bone marrow	(49)	(49)	(50)
Hyperplasia	3 (6%)	5 (10%)	4 (8%)
Hyperplasia, megakaryocyte			1 (2%)
Infiltration cellular, mast cell			1 (2%)
Necrosis	1 (2%)		
Lymph node	(2)	(3)	(7)
Congestion			1 (14%)
Iliac, hyperplasia			2 (29%)
Iliac, infiltration cellular, plasma cell	1 (50%)		
Iliac, pigmentation		1 (33%)	
Renal, hyperplasia			2 (29%)
Lymph node, bronchial	(26)	(27)	(32)
Hyperplasia		1 (4%)	2 (6%)
Lymph node, mandibular	(34)	(31)	(32)
Hyperplasia	1 (3%)	1 (3%)	
Infiltration cellular, mast cell			1 (3%)
Lymph node, mesenteric	(48)	(46)	(44)
Angiectasis	3 (6%)		4 (9%)
Congestion	1 (2%)	2 (4%)	
Hematopoietic cell proliferation	2 (4%)		
Hemorrhage		1 (2%)	
Hyperplasia	2 (4%)	2 (4%)	3 (7%)
Lymph node, mediastinal	(37)	(39)	(43)
Hyperplasia	1 (3%)	5 (13%)	5 (12%)
Spleen	(49)	(49)	(50)
Angiectasis	1 (2%)		1 (2%)
Hematopoietic cell proliferation	14 (29%)	14 (29%)	15 (30%)
Hyperplasia, lymphoid	2 (4%)	2 (4%)	1 (2%)
Infiltration cellular, mast cell			1 (2%)
Pigmentation, melanin	1 (2%)		
Thymus	(29)	(26)	(25)
Atrophy	4 (14%)	1 (4%)	5 (20%)
Integumentary System			
Skin	(49)	(50)	(50)
Inflammation, chronic active	1 (2%)	1 (2%)	
Epidermis, hyperplasia		2 (4%)	
Prepuce, inflammation, chronic active	11 (22%)	13 (26%)	16 (32%)
Subcutaneous tissue, edema		1 (2%)	
Subcutaneous tissue, hemorrhage	1 (2%)		
Subcutaneous tissue, infiltration cellular, mast cell		1 (2%)	
Subcutaneous tissue, inflammation, chronic		1 (2%)	

TABLE H4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Musculoskeletal System			
Bone	(50)	(50)	(50)
Fibrous osteodystrophy	1 (2%)	2 (4%)	1 (2%)
Nervous System			
Brain	(49)	(49)	(50)
Developmental malformation	1 (2%)		
Hemorrhage	1 (2%)		
Hydrocephalus			1 (2%)
Artery, inflammation, chronic active			1 (2%)
Respiratory System			
Larynx	(49)	(49)	(50)
Hyperplasia	4 (8%)	7 (14%)	15 (30%)
Inflammation, acute	1 (2%)		
Inflammation, chronic	1 (2%)		
Inflammation, chronic active	6 (12%)	7 (14%)	7 (14%)
Inflammation, suppurative	1 (2%)	5 (10%)	4 (8%)
Epiglottis, hyperplasia	1 (2%)	2 (4%)	2 (4%)
Epiglottis, metaplasia, squamous	2 (4%)	1 (2%)	10 (20%)
Lung	(49)	(49)	(50)
Angiectasis	1 (2%)		
Hemorrhage	1 (2%)		
Inflammation, chronic, focal		1 (2%)	
Alveolar epithelium, hyperplasia	10 (20%)	8 (16%)	1 (2%)
Alveolar epithelium, metaplasia		48 (98%)	47 (94%)
Alveolus, infiltration cellular, histiocyte	3 (6%)	40 (82%)	41 (82%)
Bronchiole, metaplasia			1 (2%)
Bronchiole, necrosis			1 (2%)
Perivascular, infiltration cellular	1 (2%)		3 (6%)
Nose	(49)	(48)	(49)
Lateral wall, degeneration, hyaline	2 (4%)	48 (100%)	49 (100%)
Lateral wall, fibrosis		8 (17%)	43 (88%)
Lateral wall, hyperplasia	2 (4%)	33 (69%)	45 (92%)
Lateral wall, inflammation, suppurative	1 (2%)	38 (79%)	46 (94%)
Lateral wall, metaplasia, squamous	1 (2%)	2 (4%)	20 (41%)
Nasolacrimal duct, inflammation, suppurative	2 (4%)	2 (4%)	2 (4%)
Olfactory epithelium, atrophy	4 (8%)	4 (8%)	18 (37%)
Trachea	(49)	(49)	(49)
Hyperplasia	1 (2%)		
Metaplasia, squamous			1 (2%)
Special Senses System			
Eye	(3)	(1)	(1)
Inflammation	2 (67%)	1 (100%)	1 (100%)

TABLE H4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Urinary System			
Kidney	(49)	(49)	(50)
Cyst	4 (8%)	1 (2%)	3 (6%)
Hydronephrosis		1 (2%)	
Infarct	1 (2%)		1 (2%)
Mineralization		2 (4%)	
Nephropathy	40 (82%)	43 (88%)	37 (74%)
Cortex, inflammation, suppurative		2 (4%)	3 (6%)
Papilla, inflammation, suppurative	3 (6%)	6 (12%)	6 (12%)
Papilla, necrosis	1 (2%)		
Pelvis, dilatation	2 (4%)		1 (2%)
Renal tubule, hyperplasia	1 (2%)		
Urinary bladder	(47)	(49)	(48)
Calculus gross observation		3 (6%)	
Inflammation, chronic active	2 (4%)	1 (2%)	2 (4%)
Inflammation, suppurative	1 (2%)	5 (10%)	5 (10%)
Transitional epithelium, hyperplasia			1 (2%)

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

APPENDIX I
SUMMARY OF LESIONS IN FEMALE MICE IN THE LIFETIME INHALATION STUDY OF OZONE

TABLE I1 Summary of the Incidence of Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone 242

TABLE I2 Individual Animal Respiratory System Tumor Pathology of Female Mice in the Lifetime Inhalation Study of Ozone 246

TABLE I3 Statistical Analysis of Primary Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone 249

TABLE I4 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Lifetime Inhalation Study of Ozone 255

TABLE I1
Summary of the Incidence of Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone[a]

	0 ppm	0.5 ppm	1.0 ppm
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	34	25	33
Natural deaths	7	13	7
Survivors			
Terminal sacrifice	9	12	10
Animals examined microscopically	50	50	50
Alimentary System			
Gallbladder	(43)	(38)	(44)
Histiocytic sarcoma			1 (2%)
Sarcoma, metastatic, skin	1 (2%)		
Intestine large, cecum	(45)	(43)	(47)
Leiomyosarcoma			1 (2%)
Intestine small, duodenum	(45)	(41)	(46)
Peyer's patch, histiocytic sarcoma			1 (2%)
Intestine small, jejunum	(44)	(42)	(47)
Intestine small, ileum	(45)	(43)	(46)
Carcinoma		1 (2%)	
Liver	(49)	(50)	(50)
Hemangiosarcoma		1 (2%)	2 (4%)
Hepatocellular carcinoma	15 (31%)	15 (30%)	6 (12%)
Hepatocellular carcinoma, multiple	4 (8%)	3 (6%)	2 (4%)
Hepatocellular adenoma	11 (22%)	12 (24%)	9 (18%)
Hepatocellular adenoma, multiple	2 (4%)	4 (8%)	4 (8%)
Hepatocholangiocarcinoma		1 (2%)	
Histiocytic sarcoma	3 (6%)		2 (4%)
Osteosarcoma, metastatic, bone		1 (2%)	
Mesentery	(13)	(5)	(10)
Hemangiosarcoma		1 (20%)	
Histiocytic sarcoma			1 (10%)
Sarcoma, metastatic, skin	1 (8%)		
Pancreas	(48)	(48)	(49)
Histiocytic sarcoma	1 (2%)		
Sarcoma, metastatic, skin	1 (2%)		
Salivary glands	(50)	(48)	(49)
Sarcoma	1 (2%)		
Stomach, forestomach	(48)	(50)	(49)
Sarcoma, metastatic, skin	1 (2%)		
Squamous cell papilloma	1 (2%)		
Stomach, glandular	(48)	(49)	(48)
Histiocytic sarcoma			1 (2%)
Sarcoma, metastatic, skin	1 (2%)		
Tooth	(1)		
Adamantinoma malignant	1 (100%)		
Cardiovascular System			
Heart	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)	

TABLE I1
Summary of the Incidence of Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Endocrine System			
Adrenal cortex	(48)	(49)	(50)
Adenoma			1 (2%)
Histiocytic sarcoma			1 (2%)
Capsule, adenoma	1 (2%)		
Adrenal medulla	(48)	(49)	(50)
Pheochromocytoma malignant			1 (2%)
Pheochromocytoma benign	2 (4%)	2 (4%)	1 (2%)
Islets, pancreatic	(47)	(47)	(48)
Adenoma	1 (2%)		
Pituitary gland	(48)	(48)	(49)
Pars distalis, adenoma	19 (40%)	11 (23%)	12 (24%)
Pars distalis, carcinoma		1 (2%)	
Pars intermedia, adenoma	1 (2%)		3 (6%)
Thyroid gland	(49)	(49)	(50)
Follicular cell, adenoma		1 (2%)	1 (2%)
Follicular cell, adenoma, multiple	2 (4%)		
General Body System			
None			
Genital System			
Ovary	(49)	(48)	(50)
Cystadenoma	4 (8%)	2 (4%)	2 (4%)
Granulosa cell tumor benign		1 (2%)	
Hemangioma	1 (2%)		2 (4%)
Histiocytic sarcoma	2 (4%)		2 (4%)
Luteoma	1 (2%)	1 (2%)	1 (2%)
Uterus	(49)	(50)	(50)
Adenoma	1 (2%)		
Fibroma	1 (2%)		
Hemangioma	1 (2%)		2 (4%)
Hemangiosarcoma		1 (2%)	
Histiocytic sarcoma	3 (6%)		1 (2%)
Leiomyoma			1 (2%)
Polyp stromal	3 (6%)	2 (4%)	6 (12%)
Polyp stromal, multiple	1 (2%)		
Hematopoietic System			
Bone marrow	(49)	(49)	(50)
Hemangiosarcoma	1 (2%)	3 (6%)	1 (2%)
Histiocytic sarcoma	2 (4%)		1 (2%)
Lymph node	(15)	(6)	(9)
Iliac, histiocytic sarcoma	2 (13%)		
Pancreatic, histiocytic sarcoma	1 (7%)		
Renal, histiocytic sarcoma	1 (7%)		1 (11%)
Lymph node, bronchial	(36)	(32)	(33)
Hepatocholangiocarcinoma, metastatic, liver		1 (3%)	
Histiocytic sarcoma	1 (3%)		1 (3%)

TABLE I1
Summary of the Incidence of Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Hematopoietic System (continued)			
Lymph node, mandibular	(38)	(40)	(41)
Histiocytic sarcoma	2 (5%)		
Lymph node, mesenteric	(45)	(48)	(44)
Histiocytic sarcoma	3 (7%)		1 (2%)
Lymph node, mediastinal	(43)	(36)	(38)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver		1 (3%)	
Histiocytic sarcoma	2 (5%)		1 (3%)
Spleen	(49)	(50)	(50)
Hemangiosarcoma	2 (4%)	3 (6%)	3 (6%)
Histiocytic sarcoma	2 (4%)		1 (2%)
Sarcoma, metastatic, skin	1 (2%)		
Thymus	(35)	(35)	(33)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (3%)		
Integumentary System			
Mammary gland	(50)	(50)	(50)
Adenoma	1 (2%)		
Carcinoma	3 (6%)	2 (4%)	2 (4%)
Carcinoma, multiple			1 (2%)
Skin	(50)	(50)	(50)
Schwannoma malignant		1 (2%)	
Subcutaneous tissue, hemangiosarcoma	1 (2%)	2 (4%)	
Subcutaneous tissue, histiocytic sarcoma	1 (2%)		1 (2%)
Subcutaneous tissue, sarcoma	1 (2%)	2 (4%)	5 (10%)
Subcutaneous tissue, sarcoma, multiple	1 (2%)		1 (2%)
Musculoskeletal System			
Bone	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)
Osteosarcoma	2 (4%)	1 (2%)	
Skeletal muscle			(2)
Histiocytic sarcoma			1 (50%)
Rhabdomyosarcoma			1 (50%)
Nervous System			
Brain	(49)	(49)	(50)
Carcinoma, metastatic, harderian gland	1 (2%)		
Carcinoma, metastatic, pituitary gland		1 (2%)	
Histiocytic sarcoma	1 (2%)		
Respiratory System			
Lung	(50)	(49)	(50)
Adamantinoma malignant, metastatic, tooth	1 (2%)		
Alveolar/bronchiolar adenoma	3 (6%)	3 (6%)	10 (20%)
Alveolar/bronchiolar adenoma, multiple			1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)	5 (10%)	2 (4%)

TABLE I1
Summary of the Incidence of Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Respiratory System (continued)			
Lung (continued)	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, multiple	2 (4%)		
Carcinoma, metastatic, harderian gland	1 (2%)	1 (2%)	
Hepatocellular carcinoma, metastatic, liver	2 (4%)	5 (10%)	1 (2%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)	
Histiocytic sarcoma	2 (4%)		2 (4%)
Osteosarcoma, metastatic, bone	1 (2%)	1 (2%)	
Sarcoma, metastatic, skin			1 (2%)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	2 (4%)		
Mediastinum, hemangiosarcoma		1 (2%)	
Mediastinum, hepatocholangiocarcinoma, metastatic, liver		1 (2%)	
Mediastinum, osteosarcoma, metastatic, bone		1 (2%)	
Mediastinum, sarcoma, metastatic, skin		1 (2%)	
Nose	(50)	(49)	(50)
Adenoma			1 (2%)
Carcinoma, metastatic, harderian gland	1 (2%)		
Special Senses System			
Harderian gland	(5)	(5)	(3)
Adenoma	4 (80%)	4 (80%)	2 (67%)
Carcinoma	1 (20%)	1 (20%)	1 (33%)
Urinary System			
Kidney	(49)	(49)	(50)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)	
Histiocytic sarcoma	1 (2%)		1 (2%)
Urinary bladder	(47)	(48)	(46)
Hemangioma	1 (2%)		
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Histiocytic sarcoma	4 (8%)		3 (6%)
Lymphoma malignant	13 (26%)	12 (24%)	13 (26%)
Neoplasm Summary			
Total animals with primary neoplasms[c]	48	49	47
Total primary neoplasms	115	100	105
Total animals with benign neoplasms	39	32	35
Total benign neoplasms	62	43	59
Total animals with malignant neoplasms	39	39	34
Total malignant neoplasms	53	57	46
Total animals with metastatic neoplasms	8	10	2
Total metastatic neoplasms	17	17	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 I2
Individual Animal Respiratory System Tumor Pathology of Female Mice in the Lifetime Inhalation Study of Ozone: 0 ppm

Number of Days on Study	4	4	5	5	5	5	5	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	8
	0	0	2	2	3	4	5	2	7	1	2	2	2	2	4	4	5	7	7	9	9	9	9	9	0
	3	8	1	7	7	7	6	1	8	7	1	1	2	4	9	9	4	5	8	0	1	1	1	6	1
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
	0	4	2	3	1	5	0	0	3	4	2	2	4	2	1	1	4	3	0	1	1	3	4	4	2
	7	9	1	6	5	0	8	5	2	3	7	9	4	2	7	8	0	0	1	2	1	3	1	2	3
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adamantinoma malignant, metastatic, tooth		X																							
Alveolar/bronchiolar adenoma												X		X											
Alveolar/bronchiolar carcinoma																									
Alveolar/bronchiolar carcinoma, multiple			X		X																				
Carcinoma, metastatic, harderian gland															X										
Hepatocellular carcinoma, metastatic, liver												X											X		
Histiocytic sarcoma																									
Osteosarcoma, metastatic, bone	X																								
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung			X		X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, harderian gland															X										
Trachea	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+

Number of Days on Study	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	9	9	9	9	9	9	9	9	9	
	0	2	2	2	4	4	4	4	4	5	6	6	6	7	8	8	1	1	1	1	1	1	1	1	1	
	5	4	6	6	0	0	1	7	9	7	0	5	9	5	3	4	1	1	1	2	2	2	2	2	2	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	Total
	0	4	2	4	0	0	2	1	3	4	0	3	1	2	3	3	0	2	3	1	1	1	2	3	4	Tissues/
	2	8	5	7	4	9	0	6	7	6	3	8	9	4	4	1	6	6	5	0	3	4	8	9	5	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adamantinoma malignant, metastatic, tooth																										1
Alveolar/bronchiolar adenoma																				X						3
Alveolar/bronchiolar carcinoma														X												1
Alveolar/bronchiolar carcinoma, multiple																										2
Carcinoma, metastatic, harderian gland																										1
Hepatocellular carcinoma, metastatic, liver																										2
Histiocytic sarcoma			X																				X			2
Osteosarcoma, metastatic, bone																										1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, harderian gland																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

+: Tissue examined microscopically X: Lesion present A: Autolysis precludes examination

TABLE I2
Individual Animal Respiratory System Tumor Pathology of Female Mice in the Lifetime Inhalation Study of Ozone: 0.5 ppm

Number of Days on Study	5	5	5	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	8	8	8	8	
	4	7	9	1	1	2	2	7	9	2	2	5	5	6	6	7	8	8	8	9	9	0	0	1	2	
	4	1	5	6	6	1	3	8	1	1	6	0	4	3	6	5	4	5	6	2	2	5	7	7	1	
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	1	0	3	3	4	3	4	2	2	4	2	4	2	3	4	2	1	0	3	1	4	0	1	4	0	
	7	1	2	3	9	1	4	1	4	8	6	3	5	4	6	0	9	5	8	4	7	2	1	5	4	
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma					X											X										
Alveolar/bronchiolar carcinoma										X																
Carcinoma, metastatic, harderian gland																										
Hepatocellular carcinoma, metastatic, liver									X		X			X												
Hepatocholangiocarcinoma, metastatic, liver	X																									
Osteosarcoma, metastatic, bone																			X							
Mediastinum, hemangiosarcoma																										
Mediastinum, hepatocholangiocarcinoma, metastatic, liver	X																									
Mediastinum, osteosarcoma, metastatic, bone																			X							
Mediastinum, sarcoma, metastatic, skin																									X	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	

Number of Days on Study	8	8	8	8	8	8	8	8	8	8	8	8	8	9	9	9	9	9	9	9	9	9	9	9	9	
	2	5	5	5	6	6	7	7	7	7	8	8	8	1	1	1	1	1	1	1	1	1	1	1	1	
	6	5	5	5	1	3	4	5	5	7	0	0	3	1	1	1	2	2	2	2	2	2	2	2	2	
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Total
	0	0	3	5	1	0	4	2	3	0	2	3	4	1	2	3	0	1	1	1	1	2	2	3	4	Tissues/
	8	7	5	0	5	3	2	3	0	6	2	9	0	8	9	7	9	0	2	3	6	7	8	6	1	Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma			X																							3
Alveolar/bronchiolar carcinoma				X				X												X				X		5
Carcinoma, metastatic, harderian gland															X											1
Hepatocellular carcinoma, metastatic, liver			X									X														5
Hepatocholangiocarcinoma, metastatic, liver																										1
Osteosarcoma, metastatic, bone																										1
Mediastinum, hemangiosarcoma															X											1
Mediastinum, hepatocholangiocarcinoma, metastatic, liver																										1
Mediastinum, osteosarcoma, metastatic, bone																										1
Mediastinum, sarcoma, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE I2
Individual Animal Respiratory System Tumor Pathology of Female Mice in the Lifetime Inhalation Study of Ozone: 1.0 ppm

Number of Days on Study	416	455	530	559	586	588	646	647	651	660	665	678	680	693	696	697	703	722	735	741	749	749	755	767	774	
Carcass ID Number	1338	1331	1334	1341	1336	1337	1340	1342	1337	1339	1331	1330	1335	1339	1340	1331	1332	1321	1321	1310	1324	1328	1341	1323	1318	
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma		X					X					X														
Alveolar/bronchiolar adenoma, multiple																										
Alveolar/bronchiolar carcinoma																										
Hepatocellular carcinoma, metastatic, liver																X										
Histiocytic sarcoma																	X	X								
Sarcoma, metastatic, skin				X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma																										
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Number of Days on Study	778	836	833	835	847	847	852	855	869	869	874	874	880	903	903	911	911	911	911	911	912	912	912	912	912	
Carcass ID Number	1327	1330	1336	1332	1319	1339	1329	1304	1306	1304	1305	1334	1337	1308	1303	1310	1313	1326	1343	1345	1315	1323	1340	1344	1350	Total Tissues/ Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma					X					X	X					X	X				X	X				10
Alveolar/bronchiolar adenoma, multiple									X																	1
Alveolar/bronchiolar carcinoma			X														X									2
Hepatocellular carcinoma, metastatic, liver																										1
Histiocytic sarcoma																										2
Sarcoma, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																						X				1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE I3
Statistical Analysis of Primary Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone

	0 ppm	0.5 ppm	1.0 ppm
Bone Marrow: Hemangiosarcoma			
Overall rate[a]	1/49 (2%)	3/49 (6%)	1/50 (2%)
Adjusted rate[b]	2.3%	12.0%	2.3%
Terminal rate[c]	0/9 (0%)	0/12 (0%)	0/10 (0%)
First incidence (days)	621	571	646
Life table test[d]	P=0.583N	P=0.395	P=0.753N
Logistic regression test[d]	P=0.597N	P=0.253	P=0.765
Cochran-Armitage test[d]	P=0.602N		
Fisher exact test[d]		P=0.309	P=0.747N
Harderian Gland: Adenoma			
Overall rate	4/50 (8%)	4/50 (8%)	2/50 (4%)
Adjusted rate	12.2%	22.4%	11.9%
Terminal rate	0/9 (0%)	2/12 (17%)	1/10 (10%)
First incidence (days)	521	621	530
Life table test	P=0.272N	P=0.555N	P=0.371N
Logistic regression test	P=0.272N	P=0.631	P=0.332N
Cochran-Armitage test	P=0.274N		
Fisher exact test		P=0.643N	P=0.339N
Harderian Gland: Adenoma or Carcinoma			
Overall rate	5/50 (10%)	5/50 (10%)	3/50 (6%)
Adjusted rate	14.6%	30.2%	17.1%
Terminal rate	0/9 (0%)	3/12 (25%)	1/10 (10%)
First incidence (days)	521	621	530
Life table test	P=0.289N	P=0.526N	P=0.382N
Logistic regression test	P=0.297N	P=0.629	P=0.352N
Cochran-Armitage test	P=0.297N		
Fisher exact test		P=0.630N	P=0.357N
Liver: Hepatocellular Adenoma			
Overall rate	13/49 (27%)	16/50 (32%)	13/50 (26%)
Adjusted rate	50.9%	62.4%	69.3%
Terminal rate	2/9 (22%)	4/12 (33%)	6/10 (60%)
First incidence (days)	527	616	416
Life table test	P=0.516N	P=0.565	P=0.580N
Logistic regression test	P=0.542N	P=0.413	P=0.577N
Cochran-Armitage test	P=0.519N		
Fisher exact test		P=0.353	P=0.567N
Liver: Hepatocellular Carcinoma			
Overall rate	19/49 (39%)	18/50 (36%)	8/50 (16%)
Adjusted rate	69.2%	68.2%	42.4%
Terminal rate	3/9 (33%)	6/12 (50%)	3/10 (30%)
First incidence (days)	547	691	651
Life table test	P=0.018N	P=0.264N	P=0.021N
Logistic regression test	P=0.009N	P=0.379N	P=0.011N
Cochran-Armitage test	P=0.009N		
Fisher exact test		P=0.469N	P=0.010N

TABLE I3
Statistical Analysis of Primary Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rate	27/49 (55%)	28/50 (56%)	21/50 (42%)
Adjusted rate	80.9%	85.8%	93.7%
Terminal rate	4/9 (44%)	8/12 (67%)	9/10 (90%)
First incidence (days)	527	616	416
Life table test	P=0.177N	P=0.315N	P=0.200N
Logistic regression test	P=0.124N	P=0.508N	P=0.144N
Cochran-Armitage test	P=0.113N		
Fisher exact test		P=0.545	P=0.135N
Lung: Alveolar/bronchiolar Adenoma			
Overall rate	3/50 (6%)	3/49 (6%)	11/50 (22%)
Adjusted rate	15.7%	8.9%	56.1%
Terminal rate	1/9 (11%)	0/12 (0%)	4/10 (40%)
First incidence (days)	721	616	455
Life table test	P=0.012	P=0.586N	P=0.035
Logistic regression test	P=0.009	P=0.633	P=0.020
Cochran-Armitage test	P=0.009		
Fisher exact test		P=0.651	P=0.020
Lung: Alveolar/bronchiolar Carcinoma			
Overall rate	3/50 (6%)	5/49 (10%)	2/50 (4%)
Adjusted rate	12.2%	26.4%	13.9%
Terminal rate	0/9 (0%)	2/12 (17%)	1/10 (10%)
First incidence (days)	521	721	833
Life table test	P=0.395N	P=0.494	P=0.473N
Logistic regression test	P=0.423N	P=0.328	P=0.496N
Cochran-Armitage test	P=0.421N		
Fisher exact test		P=0.346	P=0.500N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rate	6/50 (12%)	8/49 (16%)	12/50 (24%)
Adjusted rate	26.0%	33.1%	58.0%
Terminal rate	1/9 (11%)	2/12 (17%)	4/10 (40%)
First incidence (days)	521	616	455
Life table test	P=0.096	P=0.547	P=0.143
Logistic regression test	P=0.072	P=0.341	P=0.096
Cochran-Armitage test	P=0.074		
Fisher exact test		P=0.371	P=0.096
Mammary Gland: Carcinoma			
Overall rate	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted rate	20.5%	7.1%	12.5%
Terminal rate	1/9 (11%)	0/12 (0%)	0/10 (0%)
First incidence (days)	824	785	749
Life table test	P=0.582	P=0.400N	P=0.656N
Logistic regression test	P=0.580	P=0.452N	P=0.653
Cochran-Armitage test	P=0.588N		
Fisher exact test		P=0.500N	P=0.661N

TABLE I3
Statistical Analysis of Primary Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Mammary Gland: Adenoma or Carcinoma			
Overall rate	4/50 (8%)	2/50 (4%)	3/50 (6%)
Adjusted rate	22.9%	7.1%	12.5%
Terminal rate	1/9 (11%)	0/12 (0%)	0/10 (0%)
First incidence (days)	775	785	749
Life table test	P=0.437N	P=0.256N	P=0.518N
Logistic regression test	P=0.424N	P=0.298N	P=0.512N
Cochran-Armitage test	P=0.417N		
Fisher exact test		P=0.339N	P=0.500N
Ovary: Cystadenoma			
Overall rate	4/49 (8%)	2/48 (4%)	2/50 (4%)
Adjusted rate	13.1%	6.7%	20.0%
Terminal rate	0/9 (0%)	0/12 (0%)	2/10 (20%)
First incidence (days)	537	754	911 (T)
Life table test	P=0.253N	P=0.282N	P=0.337N
Logistic regression test	P=0.247N	P=0.382N	P=0.331N
Cochran-Armitage test	P=0.246N		
Fisher exact test		P=0.349N	P=0.329N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rate	19/48 (40%)	11/48 (23%)	12/49 (24%)
Adjusted rate	71.7%	50.8%	70.5%
Terminal rate	4/9 (44%)	4/12 (33%)	6/10 (60%)
First incidence (days)	547	691	680
Life table test	P=0.077N	P=0.028N	P=0.110N
Logistic regression test	P=0.072N	P=0.039N	P=0.098N
Cochran-Armitage test	P=0.064N		
Fisher exact test		P=0.061N	P=0.084N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rate	19/48 (40%)	12/48 (25%)	12/49 (24%)
Adjusted rate	71.7%	52.5%	70.5%
Terminal rate	4/9 (44%)	4/12 (33%)	6/10 (60%)
First incidence (days)	547	691	680
Life table test	P=0.080N	P=0.045N	P=0.110N
Logistic regression test	P=0.074N	P=0.063N	P=0.098N
Cochran-Armitage test	P=0.065N		
Fisher exact test		P=0.095N	P=0.084N
Pituitary Gland (Pars Intermedia): Adenoma			
Overall rate	1/48 (2%)	0/48 (0%)	3/49 (6%)
Adjusted rate	4.8%	0.0%	23.8%
Terminal rate	0/9 (0%)	0/12 (0%)	2/10 (20%)
First incidence (days)	840	–[e]	847
Life table test	P=0.187	P=0.473N	P=0.339
Logistic regression test	P=0.167	P=0.484N	P=0.302
Cochran-Armitage test	P=0.181		
Fisher exact test		P=0.500N	P=0.316

TABLE I3
Statistical Analysis of Primary Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Skin (Subcutaneous Tissue): Sarcoma			
Overall rate	2/50 (4%)	2/50 (4%)	6/50 (12%)
Adjusted rate	6.7%	5.8%	25.9%
Terminal rate	0/9 (0%)	0/12 (0%)	1/10 (10%)
First incidence (days)	754	595	455
Life table test	P=0.082	P=0.655N	P=0.146
Logistic regression test	P=0.084	P=0.659	P=0.136
Cochran-Armitage test	P=0.080		
Fisher exact test		P=0.691N	P=0.134
Spleen: Hemangiosarcoma			
Overall rate	2/49 (4%)	3/50 (6%)	3/50 (6%)
Adjusted rate	5.5%	15.8%	21.8%
Terminal rate	0/9 (0%)	1/12 (8%)	2/10 (20%)
First incidence (days)	621	571	646
Life table test	P=0.428	P=0.574	P=0.499
Logistic regression test	P=0.422	P=0.474	P=0.509
Cochran-Armitage test	P=0.421		
Fisher exact test		P=0.510	P=0.510
Uterus: Stromal Polyp			
Overall rate	4/50 (8%)	2/50 (4%)	6/50 (12%)
Adjusted rate	28.6%	6.6%	20.6%
Terminal rate	2/9 (22%)	0/12 (0%)	0/10 (0%)
First incidence (days)	805	792	693
Life table test	P=0.274	P=0.261N	P=0.363
Logistic regression test	P=0.283	P=0.292N	P=0.359
Cochran-Armitage test	P=0.290		
Fisher exact test		P=0.339N	P=0.370
All Organs: Hemangioma			
Overall rate	3/50 (6%)	0/50 (0%)	4/50 (8%)
Adjusted rate	16.3%	0.0%	26.6%
Terminal rate	0/9 (0%)	0/12 (0%)	2/10 (20%)
First incidence (days)	749	–	665
Life table test	P=0.430	P=0.104N	P=0.533
Logistic regression test	P=0.395	P=0.110N	P=0.487
Cochran-Armitage test	P=0.406		
Fisher exact test		P=0.121N	P=0.500
All Organs: Hemangiosarcoma			
Overall rate	2/50 (4%)	7/50 (14%)	5/50 (10%)
Adjusted rate	5.5%	33.1%	30.6%
Terminal rate	0/9 (0%)	2/12 (17%)	2/10 (20%)
First incidence (days)	621	571	646
Life table test	P=0.215	P=0.158	P=0.229
Logistic regression test	P=0.187	P=0.086	P=0.214
Cochran-Armitage test	P=0.195		
Fisher exact test		P=0.080	P=0.218

TABLE I3
Statistical Analysis of Primary Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
All Organs: Hemangioma or Hemangiosarcoma			
Overall rate	5/50 (10%)	7/50 (14%)	9/50 (18%)
Adjusted rate	20.9%	33.1%	52.3%
Terminal rate	0/9 (0%)	2/12 (17%)	4/10 (40%)
First incidence (days)	621	571	646
Life table test	P=0.185	P=0.524	P=0.227
Logistic regression test	P=0.145	P=0.401	P=0.183
Cochran-Armitage test	P=0.157		
Fisher exact test		P=0.380	P=0.194
All Organs: Histiocytic Sarcoma			
Overall rate	4/50 (8%)	0/50 (0%)	3/50 (6%)
Adjusted rate	25.9%	0.0%	8.1%
Terminal rate	1/9 (11%)	0/12 (0%)	0/10 (0%)
First incidence (days)	826	–	647
Life table test	P=0.394N	P=0.038N	P=0.483N
Logistic regression test	P=0.407N	P=0.045N	P=0.504N
Cochran-Armitage test	P=0.406N		
Fisher exact test		P=0.059N	P=0.500N
All Organs: Malignant Lymphoma or Histiocytic Sarcoma			
Overall rate	17/50 (34%)	12/50 (24%)	16/50 (32%)
Adjusted rate	60.7%	42.7%	52.2%
Terminal rate	2/9 (22%)	2/12 (17%)	1/10 (10%)
First incidence (days)	527	616	647
Life table test	P=0.489N	P=0.117N	P=0.525N
Logistic regression test	P=0.455N	P=0.171N	P=0.501N
Cochran-Armitage test	P=0.457N		
Fisher exact test		P=0.189N	P=0.500N
All Organs (Malignant Lymphoma): Histiocytic or Lymphocytic			
Overall rate	13/50 (26%)	12/50 (24%)	13/50 (26%)
Adjusted rate	46.2%	42.7%	48.0%
Terminal rate	1/9 (11%)	2/12 (17%)	1/10 (10%)
First incidence (days)	527	616	651
Life table test	P=0.507	P=0.358N	P=0.542
Logistic regression test	P=0.547	P=0.501N	P=0.593
Cochran-Armitage test	P=0.546N		
Fisher exact test		P=0.500N	P=0.590N
All Organs: Benign Neoplasms			
Overall rate	39/50 (78%)	32/50 (64%)	35/50 (70%)
Adjusted rate	94.0%	87.6%	100.0%
Terminal rate	7/9 (78%)	8/12 (67%)	10/10 (100%)
First incidence (days)	521	616	416
Life table test	P=0.303N	P=0.055N	P=0.345N
Logistic regression test	P=0.229N	P=0.066N	P=0.253N
Cochran-Armitage test	P=0.221N		
Fisher exact test		P=0.093N	P=0.247N

TABLE I3
Statistical Analysis of Primary Neoplasms in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
All Organs: Malignant Neoplasms			
Overall rate	39/50 (78%)	39/50 (78%)	34/50 (68%)
Adjusted rate	91.6%	94.3%	85.2%
Terminal rate	6/9 (67%)	10/12 (83%)	5/10 (50%)
First incidence (days)	403	544	455
Life table test	P=0.292N	P=0.241N	P=0.320N
Logistic regression test	P=0.151N	P=0.580	P=0.183N
Cochran-Armitage test	P=0.150N		
Fisher exact test		P=0.595N	P=0.184N
All Organs: Benign or Malignant Neoplasms			
Overall rate	48/50 (96%)	49/50 (98%)	47/50 (94%)
Adjusted rate	97.9%	100.0%	100.0%
Terminal rate	8/9 (89%)	12/12 (100%)	10/10 (100%)
First incidence (days)	403	544	416
Life table test	P=0.473N	P=0.232N	P=0.501N
Logistic regression test	P=0.399N	P=0.470	P=0.497N
Cochran-Armitage test	P=0.399N		
Fisher exact test		P=0.500	P=0.500N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for bone marrow, liver, lung, ovary, pituitary gland, and spleen; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE I4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Lifetime Inhalation Study of Ozone[a]

	0 ppm	0.5 ppm	1.0 ppm
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Moribund	34	25	33
Natural deaths	7	13	7
Survivors			
Terminal sacrifice	9	12	10
Animals examined microscopically	50	50	50
Alimentary System			
Gallbladder	(43)	(38)	(44)
Inflammation, suppurative	1 (2%)		
Epithelium, hyperplasia	2 (5%)		
Intestine small, duodenum	(45)	(41)	(46)
Necrosis	1 (2%)		1 (2%)
Epithelium, hyperplasia	1 (2%)		
Peyer's patch, hyperplasia			1 (2%)
Liver	(49)	(50)	(50)
Angiectasis	1 (2%)		1 (2%)
Basophilic focus	1 (2%)		3 (6%)
Clear cell focus	1 (2%)		
Degeneration, fatty		1 (2%)	
Eosinophilic focus	3 (6%)	6 (12%)	3 (6%)
Hematopoietic cell proliferation	4 (8%)		4 (8%)
Inflammation, chronic	2 (4%)	4 (8%)	1 (2%)
Necrosis	7 (14%)	3 (6%)	3 (6%)
Pigmentation	2 (4%)	2 (4%)	1 (2%)
Vacuolization cytoplasmic, focal	1 (2%)		
Bile duct, cyst	1 (2%)	2 (4%)	
Bile duct, hyperplasia	1 (2%)		1 (2%)
Centrilobular, degeneration	1 (2%)		
Centrilobular, necrosis	1 (2%)	1 (2%)	
Hepatocyte, atrophy	1 (2%)		
Mesentery	(13)	(5)	(10)
Artery, inflammation	1 (8%)		
Fat, inflammation, chronic	1 (8%)		
Fat, necrosis	9 (69%)	2 (40%)	4 (40%)
Lymphatic, angiectasis			1 (10%)
Pancreas	(48)	(48)	(49)
Atrophy	5 (10%)	1 (2%)	3 (6%)
Basophilic focus			5 (10%)
Inflammation	2 (4%)		1 (2%)
Duct, cyst	2 (4%)	1 (2%)	2 (4%)
Stomach, forestomach	(48)	(50)	(49)
Inflammation, suppurative	2 (4%)	3 (6%)	3 (6%)
Necrosis	1 (2%)	1 (2%)	1 (2%)
Epithelium, hyperplasia	3 (6%)	2 (4%)	6 (12%)
Stomach, glandular	(48)	(49)	(48)
Inflammation, acute		1 (2%)	
Mineralization			1 (2%)
Necrosis	1 (2%)	1 (2%)	
Epithelium, hyperplasia			1 (2%)

TABLE I4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Cardiovascular System			
Heart	(50)	(50)	(50)
Cardiomyopathy	37 (74%)	38 (76%)	36 (72%)
Inflammation, suppurative			1 (2%)
Necrosis	1 (2%)		
Pigmentation, hemosiderin			1 (2%)
Endocrine System			
Adrenal cortex	(48)	(49)	(50)
Angiectasis	1 (2%)		
Hyperplasia	3 (6%)	2 (4%)	3 (6%)
Hypertrophy	6 (13%)	5 (10%)	6 (12%)
Capsule, hyperplasia		1 (2%)	2 (4%)
Adrenal medulla	(48)	(49)	(50)
Hyperplasia	4 (8%)	3 (6%)	4 (8%)
Islets, pancreatic	(47)	(47)	(48)
Hyperplasia	1 (2%)		
Parathyroid gland	(36)	(28)	(29)
Hyperplasia	1 (3%)		
Pituitary gland	(48)	(48)	(49)
Pars distalis, hyperplasia	11 (23%)	14 (29%)	16 (33%)
Pars intermedia, hyperplasia	1 (2%)	2 (4%)	1 (2%)
Pars intermedia, hypertrophy			1 (2%)
Thyroid gland	(49)	(49)	(50)
Follicular cell, hyperplasia	22 (45%)	24 (49%)	30 (60%)
General Body System			
None			
Genital System			
Clitoral gland	(45)	(44)	(42)
Inflammation, chronic active	1 (2%)		2 (5%)
Ovary	(49)	(48)	(50)
Angiectasis	3 (6%)	2 (4%)	1 (2%)
Atrophy	28 (57%)	33 (69%)	30 (60%)
Cyst	23 (47%)	18 (38%)	16 (32%)
Inflammation, chronic	1 (2%)		1 (2%)
Necrosis		1 (2%)	
Germinal epithelium, hyperplasia	2 (4%)	4 (8%)	
Interstitial cell, hyperplasia			2 (4%)
Uterus	(49)	(50)	(50)
Angiectasis	4 (8%)	3 (6%)	2 (4%)
Cyst	6 (12%)	2 (4%)	9 (18%)
Decidual reaction			1 (2%)
Hemorrhage		1 (2%)	
Hydrometra	3 (6%)	5 (10%)	1 (2%)
Inflammation, chronic			1 (2%)
Inflammation, suppurative		1 (2%)	
Thrombosis	2 (4%)		
Endometrium, hyperplasia	1 (2%)	2 (4%)	
Myometrium, hyperplasia	1 (2%)		

TABLE I4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Hematopoietic System			
Bone marrow	(49)	(49)	(50)
Angiectasis	2 (4%)		
Hyperplasia	3 (6%)	1 (2%)	4 (8%)
Lymph node	(15)	(6)	(9)
Hyperplasia	1 (7%)		
Iliac, angiectasis	2 (13%)		
Iliac, hyperplasia	1 (7%)		
Lumbar, congestion	1 (7%)		
Lumbar, hyperplasia			1 (11%)
Renal, congestion	1 (7%)		
Renal, hyperplasia			1 (11%)
Lymph node, bronchial	(36)	(32)	(33)
Hyperplasia	1 (3%)	1 (3%)	1 (3%)
Infiltration cellular, plasma cell	1 (3%)		
Infiltration cellular, histiocyte	1 (3%)		
Lymph node, mandibular	(38)	(40)	(41)
Hematopoietic cell proliferation		1 (3%)	
Hyperplasia	2 (5%)	4 (10%)	3 (7%)
Necrosis	1 (3%)		
Lymph node, mesenteric	(45)	(48)	(44)
Angiectasis			1 (2%)
Congestion		2 (4%)	
Hematopoietic cell proliferation		1 (2%)	1 (2%)
Hyperplasia	1 (2%)	1 (2%)	
Infiltration cellular, histiocyte		1 (2%)	
Lymph node, mediastinal	(43)	(36)	(38)
Hyperplasia	5 (12%)	9 (25%)	1 (3%)
Infiltration cellular, histiocyte	2 (5%)		
Spleen	(49)	(50)	(50)
Hematopoietic cell proliferation	15 (31%)	19 (38%)	21 (42%)
Hyperplasia, histiocytic		1 (2%)	
Hyperplasia, lymphoid	6 (12%)	9 (18%)	4 (8%)
Infiltration cellular, histiocyte		1 (2%)	
Thymus	(35)	(35)	(33)
Atrophy	2 (6%)	3 (9%)	1 (3%)
Hyperplasia, lymphoid	1 (3%)		2 (6%)
Integumentary System			
Mammary gland	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	3 (6%)
Skin	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)		
Inflammation, suppurative		1 (2%)	
Vulva, inflammation, suppurative			1 (2%)

TABLE I4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Musculoskeletal System			
Bone	(50)	(50)	(50)
Chondradysplasia			1 (2%)
Fibrous osteodystrophy	15 (30%)	18 (36%)	14 (28%)
Fracture	1 (2%)		
Cranium, fracture	1 (2%)		
Cranium, myelofibrosis		1 (2%)	
Femur, myelofibrosis		1 (2%)	
Nervous System			
Brain	(49)	(49)	(50)
Angiectasis	1 (2%)		
Hydrocephalus			1 (2%)
Meninges, infiltration cellular	2 (4%)	2 (4%)	2 (4%)
Peripheral nerve	(1)		
Degeneration	1 (100%)		
Spinal cord	(1)		
Degeneration	1 (100%)		
Respiratory System			
Larynx	(50)	(49)	(50)
Hyperplasia	13 (26%)	11 (22%)	24 (48%)
Inflammation, chronic active	4 (8%)		
Inflammation, suppurative	5 (10%)	4 (8%)	10 (20%)
Necrosis			2 (4%)
Epiglottis, hyperplasia			4 (8%)
Epiglottis, metaplasia, squamous	2 (4%)	2 (4%)	19 (38%)
Lung	(50)	(49)	(50)
Congestion, chronic			2 (4%)
Hemorrhage	1 (2%)	2 (4%)	1 (2%)
Inflammation, chronic, focal	1 (2%)		
Alveolar epithelium, hyperplasia	3 (6%)	1 (2%)	3 (6%)
Alveolar epithelium, metaplasia		43 (88%)	50 (100%)
Alveolus, infiltration cellular, histiocyte	5 (10%)	39 (80%)	45 (90%)
Mediastinum, angiectasis		1 (2%)	
Mediastinum, necrosis			1 (2%)
Perivascular, infiltration cellular	6 (12%)		1 (2%)
Nose	(50)	(49)	(50)
Artery, inflammation		1 (2%)	
Lateral wall, degeneration, hyaline		49 (100%)	50 (100%)
Lateral wall, fibrosis	1 (2%)	23 (47%)	48 (96%)
Lateral wall, hyperplasia	1 (2%)	42 (86%)	47 (94%)
Lateral wall, inflammation, suppurative	3 (6%)	44 (90%)	50 (100%)
Lateral wall, metaplasia, squamous	2 (4%)	3 (6%)	28 (56%)
Lateral wall, necrosis			1 (2%)
Nasolacrimal duct, inflammation, suppurative	2 (4%)	1 (2%)	
Olfactory epithelium, atrophy	9 (18%)	23 (47%)	40 (80%)
Turbinate, hyperplasia	1 (2%)		
Turbinate, inflammation, suppurative	1 (2%)		
Trachea	(48)	(48)	(50)
Metaplasia, squamous			1 (2%)
Mineralization			1 (2%)
Necrosis			1 (2%)

TABLE I4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Lifetime Inhalation Study of Ozone (continued)

	0 ppm	0.5 ppm	1.0 ppm
Special Senses System			
Ear		(1)	
Inflammation, granulomatous		1 (100%)	
Urinary System			
Kidney	(49)	(49)	(50)
Fibrosis, focal	1 (2%)		
Glomerulosclerosis			2 (4%)
Hydronephrosis	1 (2%)		2 (4%)
Infarct		1 (2%)	
Karyomegaly			2 (4%)
Metaplasia, osseous	4 (8%)	1 (2%)	5 (10%)
Mineralization			1 (2%)
Nephropathy	24 (49%)	27 (55%)	30 (60%)
Cortex, inflammation, suppurative			1 (2%)
Pelvis, dilatation		1 (2%)	
Renal tubule, hyperplasia		2 (4%)	
Urinary bladder	(47)	(48)	(46)
Inflammation, suppurative	1 (2%)		

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

APPENDIX J
GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL 262
RESULTS 262
TABLE J1 Mutagenicity of Ozone in *Salmonella typhimurium* 263

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by McGregor *et al.* (1989) for the testing of gases, with modifications as described in Dillon *et al.* (1992). Each *Salmonella typhimurium* tester strain (TA98, TA100, TA102, TA104, or TA1535), either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male F344/N rat liver), was added to top agar and used to overlay Vogel-Bonner plates (three plates per concentration). Plates, with lids slightly raised to facilitate ozone circulation, were stacked in glass jars equipped with tapped, ground glass lids. Ozone was produced using an Ozone Generator, Type GLX (Argentox, Hamburg, Germany), operating via an electrical discharge in dry oxygen. Different concentrations of ozone were achieved by varying the flow rate of oxygen and the voltage. To expose the cells, generator voltage was applied to the oxygen flow for 5 minutes and the jars were sealed to maintain ozone atmospheres for an additional 30 minutes. Residual ozone was purged with air after this 30 minute exposure period. Plates were incubated at 37° C for 2 days in the jars and then for 1 day outside the jars. Histidine-independent mutant colonies arising on these plates were counted with a Biotran III colony counter.

The parametric method of Dunnett (1955), involving calculation of Student's *t*-statistic, was used to determine the significance of the mean counts at each individual dose level. To analyze dose responses, a nonparametric ranking procedure was used (Wahrendorf *et al.*, 1985).

RESULTS

Concurrent dosimetry was conducted with each trial because, as shown in Table J1, identical voltage and oxygen flow parameters did not ensure identical ozone concentrations. Generation of ozone from oxygen was not 100% efficient and some residual oxygen was presumably present in the exposure jar atmospheres, but the amount could not be quantitated. Therefore, statistical analyses presented in Table 1 are from comparisons with air controls only, although the data for the oxygen controls are included. Comparison of the individual dose points to the oxygen control values reduced the significance of some of the responses, but did not change a mutagenic response to a nonmutagenic response in any of the experiments (see Dillon *et al.*, 1992).

No induction of mutations was observed in experiments conducted with an oxygen flow rate of 5 L/minute with strains TA98, TA100, TA104, or TA1535 (data not shown; see Dillon *et al.*, 1992). Positive responses were obtained with strain TA102, however, in all four experiments conducted, two with oxygen flow rates of 5 L/minute and two with flow rates of 7 L/minute; the data presented in Table 1 are from the second set of experiments (Dillon *et al.*, 1992). The same voltage settings were used in all experiments. In most experiments, similar results were obtained with and without S9. The positive responses occurred at the lower voltages (100, 125, and 132 volts); higher voltages, that produced higher concentrations of ozone, resulted in increasing toxicity and decreases in the numbers of mutant colonies.

TABLE J1
Mutagenicity of Ozone in *Salmonella typhimurium*[a]

Strain	Volts	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA102	0 (air)	0	189 ± 15	222 ± 10	204 ± 7	245 ± 8
	0 (O_2)	0	217 ± 16	250 ± 27	213 ± 11	263 ± 26
	100	0.019		500 ± 86**		472 ± 34**
		0.024	549 ± 40**		599 ± 114**	
	125	0.19	584 ± 2**		632 ± 54**	
		0.22		572 ± 9**		543 ± 31**
	132	0.53	479 ± 17**		491 ± 38**	
		0.64		222 ± 8		245 ± 22
	150	1.48	222 ± 11		218 ± 17	
		1.52		214 ± 8		188 ± 13
	180[b]	3.48		100 ± 9		194 ± 24
		3.62	187 ± 7		182 ± 5	
	220[b]	7.04	0 ± 0		0 ± 0	
		7.08		73 ± 6		0 ± 0
Trial summary			Positive	Positive	Positive	Positive
Positive control[c]			752 ± 3**	512 ± 29**	1,016 ± 69**	2,307 ± 261**

** Significantly different from air controls ($P<0.01$) by Dunnett's test.
[a] The detailed protocol and these data are presented in Dillon *et al.* (1992). Flow rate of oxygen, 7 L/minute.
[b] Slight toxicity, manifested by thinning of background lawn.
[c] 2-Aminoanthracene was used in the presence of S9. In the absence of metabolic activation, mitomycin-C was tested.

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

TABLE K1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 4-Week Inhalation Study of Ozone 266
TABLE K2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 4-Week Inhalation Study of Ozone 267

TABLE K1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 4-Week Inhalation Study of Ozone[a]

	0 ppm	0.5 ppm	1 ppm
Male			
n	5	5	5
Necropsy body wt	242 ± 6	238 ± 9	224 ± 5
Heart			
Absolute	0.804 ± 0.031	0.800 ± 0.020	0.770 ± 0.019
Relative	3.32 ± 0.05	3.37 ± 0.08	3.43 ± 0.04
R. Kidney			
Absolute	0.944 ± 0.036	0.978 ± 0.033	0.870 ± 0.018
Relative	3.90 ± 0.08	4.11 ± 0.10	3.88 ± 0.05
Liver			
Absolute	10.224 ± 0.300	10.932 ± 0.343	10.072 ± 0.294
Relative	42.29 ± 0.56	45.97 ± 0.64*	44.93 ± 1.03
Lungs			
Absolute	1.282 ± 0.090	1.480 ± 0.076	1.634 ± 0.130
Relative	5.29 ± 0.27	6.22 ± 0.24	7.29 ± 0.57**
R. Testis			
Absolute	1.261 ± 0.047	1.252 ± 0.017	1.257 ± 0.022
Relative	5.21 ± 0.11	5.28 ± 0.15	5.62 ± 0.14
Thymus			
Absolute	0.480 ± 0.046	0.457 ± 0.012	0.428 ± 0.028
Relative	1.97 ± 0.15	1.93 ± 0.09	1.91 ± 0.11
Female			
n	5	5	5
Necropsy body wt	148 ± 1	155 ± 5	144 ± 3
Heart			
Absolute	0.510 ± 0.012	0.528 ± 0.015	0.536 ± 0.019
Relative	3.45 ± 0.07	3.42 ± 0.04	3.72 ± 0.11
R. Kidney			
Absolute	0.570 ± 0.008	0.624 ± 0.024	0.588 ± 0.012
Relative	3.86 ± 0.05	4.04 ± 0.07	4.09 ± 0.11
Liver			
Absolute	5.354 ± 0.046	6.088 ± 0.360	5.764 ± 0.258
Relative	36.25 ± 0.23	39.28 ± 1.24	39.99 ± 1.19*
Lungs			
Absolute	0.824 ± 0.031	0.934 ± 0.022	1.028 ± 0.054**
Relative	5.59 ± 0.25	6.06 ± 0.18	7.14 ± 0.33**
Thymus			
Absolute	0.351 ± 0.011	0.354 ± 0.011	0.302 ± 0.011*
Relative	2.37 ± 0.06	2.30 ± 0.07	2.10 ± 0.06*

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

TABLE K2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 4-Week Inhalation Study of Ozone[a]

	0 ppm	0.5 ppm	1 ppm
Male			
n	5	5	5
Necropsy body wt	31.5 ± 1.2	29.1 ± 0.7	28.9 ± 0.4
Heart			
Absolute	0.138 ± 0.007	0.144 ± 0.010	0.128 ± 0.004
Relative	4.40 ± 0.22	4.93 ± 0.26	4.43 ± 0.14
R. Kidney			
Absolute	0.260 ± 0.016	0.274 ± 0.016	0.250 ± 0.007
Relative	8.30 ± 0.53	9.39 ± 0.33	8.64 ± 0.20
Liver			
Absolute	1.468 ± 0.053	1.398 ± 0.055	1.380 ± 0.016
Relative	46.67 ± 0.89	47.97 ± 0.75	47.70 ± 0.54
Lungs			
Absolute	0.188 ± 0.005	0.182 ± 0.007	0.198 ± 0.006
Relative	6.01 ± 0.28	6.25 ± 0.16	6.85 ± 0.24*
R. Testis			
Absolute	0.110 ± 0.002	0.110 ± 0.002	0.109 ± 0.002
Relative	3.51 ± 0.14	3.80 ± 0.11	3.76 ± 0.07
Thymus			
Absolute	0.073 ± 0.007	0.058 ± 0.004	0.066 ± 0.004
Relative	2.29 ± 0.15	1.99 ± 0.13	2.29 ± 0.11
Female			
n	5	5	5
Necropsy body wt	26.7 ± 1.9	24.3 ± 0.3	25.8 ± 1.4
Heart			
Absolute	0.114 ± 0.002	0.116 ± 0.004	0.106 ± 0.002
Relative	4.36 ± 0.31	4.78 ± 0.18	4.14 ± 0.16
R. Kidney			
Absolute	0.172 ± 0.006	0.182 ± 0.007	0.178 ± 0.009
Relative	6.59 ± 0.54	7.51 ± 0.37	6.97 ± 0.47
Liver			
Absolute	1.232 ± 0.058	1.132 ± 0.027	1.226 ± 0.058
Relative	46.52 ± 1.56	46.56 ± 0.61	47.63 ± 0.68
Lungs			
Absolute	0.182 ± 0.004	0.184 ± 0.002	0.186 ± 0.010
Relative	6.93 ± 0.39	7.58 ± 0.14	7.27 ± 0.41
Thymus			
Absolute	0.082 ± 0.007	0.074 ± 0.002	0.076 ± 0.002
Relative	3.10 ± 0.23	3.04 ± 0.13	2.98 ± 0.18

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

APPENDIX L
CHEMICAL CHARACTERIZATION, DOSE FORMULATION STUDIES, AND GENERATION OF CHAMBER CONCENTRATIONS

PROCUREMENT AND CHARACTERIZATION 270
PREPARATION AND ANALYSIS OF DOSE FORMULATIONS 272
GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS 272
FIGURE L1 Infrared Absorption Spectrum of NNK 276
FIGURE L2 Nuclear Magnetic Resonance Spectrum of NNK 277
TABLE L1 Preparation and Storage of Dose Formulations in the Inhalation Studies of Ozone ... 278
TABLE L2 Results of Analysis of NNK Dose Formulations Administered to Male Rats in the 2-Year Ozone/NNK Study 279
FIGURE L3 Ozone Vapor Generation and Delivery System 280
FIGURE L4 Ozone Inhalation Exposure Chamber 281
FIGURE L5 Ozone Exposure Suite 282
FIGURE L6 Ozone Inhalation Exposure Chamber Recirculation System 283
TABLE L3 Summary of Chamber Concentrations in the 4-Week Inhalation Studies of Ozone 284
TABLE L4 Summary of Chamber Concentrations in the 2-Year Inhalation Studies of Ozone 284
TABLE L5 Summary of Chamber Concentrations in the 2-Year Inhalation Study of Ozone/NNK . 285
TABLE L6 Summary of Chamber Concentrations in the Lifetime Inhalation Studies of Ozone ... 285
FIGURE L7 Monthly Mean Concentration and Standard Deviation in the 0.12 ppm Ozone Rat Exposure Chamber for the 2-Year Study 286
FIGURE L8 Monthly Mean Concentration and Standard Deviation in the 0.5 ppm Ozone Rat Exposure Chamber for the 2-Year Study 287
FIGURE L9 Monthly Mean Concentration and Standard Deviation in the 1.0 ppm Ozone Rat Exposure Chamber for the 2-Year Study 288
FIGURE L10 Monthly Mean Concentration and Standard Deviation in the 0.12 ppm Ozone Mouse Exposure Chamber for the 2-Year Study 289
FIGURE L11 Monthly Mean Concentration and Standard Deviation in the 0.5 ppm Ozone Mouse Exposure Chamber for the 2-Year Study 290
FIGURE L12 Monthly Mean Concentration and Standard Deviation in the 1.0 ppm Ozone Mouse Exposure Chamber for the 2-Year Study 291
FIGURE L13 Monthly Mean Concentration and Standard Deviation in the 0.5 ppm Ozone Rat Exposure Chamber for the Lifetime Study 292
FIGURE L14 Monthly Mean Concentration and Standard Deviation in the 1.0 ppm Ozone Rat Exposure Chamber for the Lifetime Study 293
FIGURE L15 Monthly Mean Concentration and Standard Deviation in the 0.5 ppm Ozone Mouse Exposure Chamber for the Lifetime Study 294
FIGURE L16 Monthly Mean Concentration and Standard Deviation in the 1.0 ppm Ozone Mouse Exposure Chamber for the Lifetime Study 295
FIGURE L17 Monthly Mean Concentration and Standard Deviation in the 0.5 ppm (Vehicle) Ozone/NNK Rat Exposure Chamber for the 2-Year Study 296
FIGURE L18 Monthly Mean Concentration and Standard Deviation in the 0.5 ppm Ozone/NNK Rat Exposure Chamber for the 2-Year Study 297

CHEMICAL CHARACTERIZATION, DOSE FORMULATION STUDIES, AND GENERATION OF CHAMBER CONCENTRATIONS

PROCUREMENT AND CHARACTERIZATION

Ozone

Ultra-high purity compressed oxygen for the generation of ozone was obtained in nine lots. Lots 12636-11 and 12821-24 were manufactured by A.L. Welding Compressed Gases (Kennewick, WA). Lot 12636-11 was used throughout the 4-week studies and for part of the 2-year studies, and lot 12821-24 was used for part of the lifetime studies. Lot 12636-58 was manufactured by Alphagaz Specialty Gases, Division of Liquid Air Corporation (Denver, CO), and it was used for part of the 2-year and lifetime studies. Lots 12733-38, 12733-81, 12733-115, 12733-121, and 12733-142 were manufactured by Scott Specialty Gases (Fremont, CA), and were used for part of the 2-year and lifetime studies. Lot 12821-7 was manufactured by Linde Gases (Torrance, CA), and it was used for part of the 2-year and lifetime studies.

A certification of oxygen purity was obtained from each of the vendors, which showed that the supplied compressed oxygen purity was greater than 99.9%. The impurities were nitrogen (<40 ppm), water (<2 ppm), carbon dioxide (<2 ppm), and total hydrocarbon (1 ppm as methane). Oxygen purity was acceptable for the studies.

The cylinders of compressed oxygen were stored in the study laboratory's outdoor storage area for compressed gases at ambient temperatures. When needed, the compressed oxygen cylinders were transferred to the exposure generation room where they were fitted with pressure regulators and attached to the ozone system inlet manifold.

4-(*N*-methyl-*N*-nitrosoamino)-1-(3-pyridyl)-1-butanone

The 4-(*N*-methyl-*N*-nitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) was obtained from Chemsyn Science Laboratories (Lenexa, KS) in one lot (86-034-01-06). Identity, purity, and stability analyses were conducted by Research Triangle Institute (RTI). Reports on analyses performed in support of the NNK studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The chemical, a yellow crystalline solid, was identified as NNK by infrared, ultraviolet/visible, nuclear magnetic resonance, and mass spectroscopy. All spectra were consistent with those expected for a mixture of the two NNK geometric isomers (Z and E forms) (Figures L1 and L2).

The purity was determined by Karl Fischer water analysis, thin-layer chromatography (TLC), and high performance liquid chromatography (HPLC). TLC was performed using two systems: 1) silica gel 60 F-254 plates with chloroform:methanol (90:10) as the solvent; and 2) Whatman KC18F plates with acetonitrile:0.25 M sodium chloride (60:40) as the solvent. Visualization was accomplished with ultraviolet light (254 nm) and I_2 vapors. HPLC was performed using two systems: A) reverse phase, DuPont Zorbax C8 column using ultraviolet detection (210 nm) and a solvent system of 0.005 M pentane sulfonic acid in acetonitrile:water (85:15) at a flow rate of 2 mL/minute; B) normal phase, DuPont Zorbax CN column using ultraviolet detection (275 nm) and a solvent system of hexane:isopropanol:dimethyl formamide (95:3:2) at a flow rate of 2 mL/minute.

Karl Fischer water analysis indicated 0.57% ± 0.011% water. TLC by each system indicated one spot and no impurities. HPLC revealed no impurities and separated the two geometric isomers E (88%) and Z (12%). The overall purity was determined to be greater than 99%. Subsequent purity analyses performed by the study laboratory using gas chromatography methods also found the overall purity to be greater than 99%.

Stability studies of the bulk chemical were performed by RTI. HPLC was performed using system A described for the purity analysis. These studies indicated that NNK was stable as a bulk chemical for at least 2 weeks when stored in the dark at temperatures up to at least 26° C. To ensure stability, the bulk chemical was stored in the original container under a nitrogen blanket protected from light at approximately 5° C.

Trioctanoin

The trioctanoin was obtained from Eastman Kodak Company, (Rochester, NY) in one lot, which was assigned the lot number M061289. Midwest Research Institute (MRI) identified the chemical, a light yellow transparent liquid, as trioctanion by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure of trioctanoin.

Purity was determined by Karl Fischer water analysis, elemental analysis, titrations for acid values, saponification value, and ester value, thin layer chromatography (TLC), and gas chromatography. Karl Fischer water analysis indicated less than 0.1% water. TLC was performed on Silica gel 60A F-254 plates using two solvent systems: 1) cyclohexane: 1,4-dioxane (95:5); and 2) carbon tetrachloride: chloroform: methanol: glacial acetic acid (60:40:1:1). Visualization was accomplished with UV light (254nm) and with a spray of potassium dichromate in 40% sulfuric acid. Gas chromatography was performed with a flame ionization detector (FID) and a helium carrier gas. Two systems were used: A) 1% SP 1000 on 100/120 Supelcoport with an oven temperature program of 185° C initially then 185° to 250° C at 10° C/min; and B) DB-1 Megabore with an oven temperature program of 50° C initially then to 275° C at 10° C/min. No attempt was made to determine the relative amounts of the two isomers (1,2- and 1,3-trioctanoin).

Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for trioctanoin. Free acid titration with 0.1 N sodium hydroxide required 8.43 mg KOH per gram of trioctanoin, equivalent to 2.17% octanoic acid in trioctanoin. Saponification titration indicated a value of 357 mg KOH per gram of sample. The ester value was calculated at 93% of the theoretical value. TLC indicated a major band and a minor and three trace impurities. Gas chromatography indicated a major peak and several impurity peaks with a cumulative area of approximately 7% relative to the major peak. The largest impurity (5.1%) was identified by a gas chromatograph/mass spectrometer as dioctanoin. The study laboratory analyzed the bulk chemical for peroxide content. All of the trioctanoin used for dose preparation was found to have a peroxide content of less than 3 mEq/kg.

Stability studies of the bulk chemical were performed by MRI. Gas chromatography was performed using system A described for the triocatoin purity analysis. These studies indicated that trioctanoin was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored in containers with a nitrogen headspace at room temperature protected from light.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

NNK/Trioctanoin

Dose formulations (NNK in trioctanoin) were prepared every 3 weeks by mixing NNK with trioctanoin (Table L1). Trioctanoin was filtered through charcoal and Celite immediately before being used for dose preparation. The dose formulations were stored at 25° C for up to 3 weeks.

Stability analysis of the 0.1 mg/kg dose formulation was performed on aqueous extracts by HPLC using system A described for the NNK purity analysis with the addition of *p*-hydroxyacetophenone as the internal standard. Stability was confirmed for 3 weeks when stored at room temperature. Periodic analyses of the dose formulations were conducted at the study laboratory using the same HPLC method. The HPLC method used by the study laboratory used a different solvent ratio (water:acetonitrile, 85:15) and a different internal standard (phenol) than the method used by RTI. Further, the HPLC solvents used by the study laboratory did not contain 0.005 M pentane sulfonic acid.

Dose formulations were analyzed at the start, middle and end of the 20-week NNK exposure period. All dose formulations used for the study were within specifications except for one 0.1 mg/mL dose formulation, which was 120% of the target formulation. One 0.1 mg/mL formulation was 80% of the target concentration, and it was discarded and remixed (Table L2). All animal room samples were within 10% of the target concentrations (Table L2).

GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS

Gas Generation System: Ozone gas was generated from ≥99.9% pure oxygen using a silent arc (corona) discharge ozonator (Model O3V5-O, OREC, Phoenix, AZ)(Figure L3). The gas then passed into a distribution manifold via a main exposure on/off valve that could be operated either manually or by computer. From the manifold, it was distributed to each chamber (Model H-2000, Harford Division of Lab Products, Aberdeen, MD) (Figure L4) through pairs of metering valves and corresponding flowmeters. The ozone was delivered to each exposure chamber through these flowmeters via three-way solenoid valves located at the chamber end of the gas delivery line. This three-way valve, controlled either manually or by computer, turned the ozone to a particular chamber on or off. When the valve to a chamber was off, the ozone to that chamber was routed to the exposure system exhaust. During the exposure period, ozone was injected into the chamber inlet duct where it was diluted with conditioned chamber air to achieve the desired exposure concentrations. A diagram of the exposure suite is shown in Figure L5.

The concentration in each chamber was controlled by manually adjusting the individual chamber metering valves. The flow of ozone to each chamber was increased above its normal operating level during the startup phase by manually adjusting the flowmetering control valves. This measure was necessary due to the reactivity of the ozone with chamber surfaces, which was especially pronounced at the beginning of each exposure period.

Test Article Concentration Monitoring: Chamber concentrations were monitored using an ultraviolet spectrophotometric analyzer (Dasibi Model 1003-AH or Dasibi Model 1003-PC systems) (Glendale, CA). Initially, the UV spectrophotometric analyzer (Dasibi Model 1003-AH) was used to monitor the ozone concentration in the exposure chambers, control chamber, room, generator cabinet, and an on-line ozone standard. After approximately 14 months (2-year ozone study), or 16 months (2-year ozone/NNK study and lifetime studies) the Model 1003-AH ozone monitors were replaced with Dasibi Model 1003-PC ozone monitors/generators. This change reduced maintenance and repair costs and maintained an effective system for monitoring ozone.

For both monitoring systems, air sampled at each location was transported to the monitor by transfer lines of Teflon® tubing. Samples were directed to the ozone monitor through a set of eight computer-controlled, multiplexed Teflon valves. A sampling rate of 4 minutes per port assured that all ports were sampled approximately twice per hour.

Output of each ozone monitor (1003-AH or 1003-PC) was automatically read and recorded by the Automated Data Acquisition and Control System. Data were sent from the ozone monitors to a Hewlett-Packard (HP) 85B computer located in the exposure control room. The HP-85B computer remotely controlled the selection of the correct sample stream and the operation of each monitor. The equation for each monitor's calibration curve was contained in the HP-85B and was applied to the analog output data (voltages) transmitted by the on-line ozone monitors. The HP-85B also accumulated and printed the sample values until all positions in the eight-valve system in each room had been measured. These measurements were then sent to the executive computer for printing and storage. Each monitor was interfaced to a Dasibi Model 1003-PC ozone standard generator to assess instrument calibration drift. These standard generators also supplied an addition ozone concentration for calibration of the on-line monitors.

Each on-line monitor was calibrated by correlating the analog output of the on-line monitor with concentrations obtained using an independently calibrated, portable ozone monitor (Dasibi Model 1003-AH). Points on the calibration curve were chamber ozone reading from the portable monitor and the corresponding voltage reading obtained simultaneously from the on-line monitor. An additional calibration point was obtained by measuring the ozone output from the on-line standard generator (0.25 or 0.5 ppm).

Calibration of the portable monitor was accomplished in a fashion similar to that described above. The portable monitor was used to monitor the output from the off-line ozone standard generator (Dasibi Model 1003-PC). The output of this generator could be maintained at any desired ozone concentration in the range of 0 to 1 ppm. Points on the calibration curve for the portable monitor consisted of ozone concentration readings obtained from the standard generator and the digital readout of the portable monitor. This generator in turn was calibrated using the chemical-specific method described below.

The chemical-specific method for ozone is an adaptation of the method of Bergshoeff *et al.* (1980). Ozone was collected from the output manifold of the standard generator using a bubbler containing a pH-buffered solution of potassium iodide (KI), potassium bromide (KBr) and potassium thiosulfate. The determination of the amount of ozone collected was based on the reaction between ozone (O_3) and iodide (I^-) to yield triiodide ion (I_3^-), according to the following reaction:

$$O_3 + 3I^- + 2H^+ = I_3^- + O_2 + H_2O$$

In practice, I_3^- is formed in a buffered solution (pH 7) containing an excess of KI and KBr, and a known amount of thiosulfate ($S_2O_3^{2-}$). Immediately after it is formed, the I_3^- reacts with the thiosulfate according to the following reaction:

$$I_3^- + 2S_2O_3^{2-} = 3I^- + S_4O_6^{2-}$$

After this reaction, the excess amount of added I_3^- that remained in the solution was measured at 352 nanometers with a conventional UV/vis spectrophotometer calibrated against volumetrically prepared standards of I_3^-. The molar amounts of I_3^- and $S_2O_3^{2-}$ used in this procedure were adjusted such that the μmoles of I_3^- remaining in solution (after correcting for the blank) were equal to the number of μmoles of ozone originally collected.

This method provided an accurate and precise determination of ozone concentrations in the range from 0 to 1 ppm ozone. Moreover, the calibration of the output of the off-line generator appeared to be quite constant and reproducible over extended periods of time. If the chemical-specific method described above provided accurate results, it was expected that the slope of the calibration curve between the chemical-specific assay of ozone and the readout of the monitor in the standard generator would be unity. This was indeed observed within experimental error, which shows that the results of the calibration methods described here agree with those employed by the manufacturer (Dasibi, 1981).

Concentration Buildup and Decay: The buildup of vapor concentration in the chamber at the beginning of exposure to 90% of its final stable concentration (T_{90}) and the decay of concentration at the end of exposure to 10% (T_{10}) were measured prior to the start of each study in chambers with a full complement of mature F344/N rats and $B6C3F_1$ mice. These tests were done in conjunction with the prestart tests for the 4-week, 2-year, and 30-month ozone studies. The measurements were repeated once after the start of the 4-week, 2-year, and lifetime studies. At a chamber airflow rate of 15 air changes/hour, the theoretical value for T_{90} and T_{10} is approximately 12.5 minutes. During the buildup time, continual adjustment of the ozone flow was required to compensate for the loss of ozone in the chambers. Based on the present data a T_{90} of 12 minutes was used for the 4-week studies, and a T_{90} of 30 minutes was used for the 2-year and lifetime studies. The measurements taken during the studies were comparable to the prestart measurements, except for the 2-year rat study, in which the value of T_{90} ranged from 14 to 22 minutes, while the value for T_{10} ranged from 5 to 7 minutes.

In order to determine the persistence of the chemical in the chamber following exposure, (i.e., after terminating test article delivery), the time for the concentration to decay to less than 1% of the stable concentration was measured in the 1.0 ppm chamber. Monitoring was performed approximately every 90 days during the lifetime study when animals were present. The values were approximately 14 minutes.

Concentration Uniformity: Tests with ozone in a standard H-2000 chamber with animals present and a standard fresh air flow rate of 15 air changes per hour indicated that acceptable uniformity of the test article was not achievable.

Concentration uniformity was improved by mixing the air within the chamber with enough energy that the rate of depletion of ozone was limited primarily by the ability of the animals or other surfaces to react with the chemical and was not limited by diffusion of the chemical within the chamber. This was accomplished using a recirculation device that increased the velocity of air movement so that the mass flow of ozone past the animals was significantly greater than the removal rate of the test article. Thus, the concentration in the vicinity of the animal was not significantly different from any other location in the chamber and concentration uniformity was improved.

The configuration of the recirculation device used in this study is shown in Figure L6. A portion of the air at the exhaust of the chamber was returned to the inlet of the chamber by means of Teflon-lined tubing and a variable-speed fan. Sufficient mass flow of ozone into the chamber to overcome absorption was accomplished by increasing the concentration of the test article at the inlet of the chamber as needed.

Uniformity of ozone concentration in the exposure chambers was measured once during the 4-week studies and quarterly during the 2-year and lifetime studies. The vapor concentration was measured using the on-line ozone monitor with the automatic sampling system disabled to allow continuous monitoring from a single input line. Concentration was measured only at those front and back sampling ports where cage units contained animals.

The possible variation of test chemical concentration measured from one sample port to another during the measurement procedure is termed the total port variability (TPV) and consists of both spatial and temporal variations. Two factors contribute to the TPV. The first, the between port variability (BPV), is

the factor of interest as it represents the spatial variation of test chemical distribution within the chamber. The second factor, the within port variability (WPV), represents the temporal fluctuation of the average chemical concentration within the chamber during the time the measurements were taken.

The recirculation system provided much improved uniformity. The uniformity criterion (BPV $\leq$ 5% relative standard deviation; RSD) was met in the 4-week studies. However, the criterion was not always met in the 2-year ozone, 2-year ozone/NNK, and lifetime studies. The maximum BPV determined during the study ranged from 10.1% in the 2-year ozone mouse study, to 5.7% in the lifetime mouse study. The measurements of WPV satisfied the WPV $\leq$ 5% criterion throughout all of the studies.

Summaries of the chamber concentrations in the 4-week, 2-year ozone, 2-year ozone/NNK, and the lifetime studies are presented in Tables L3, L4, L5 and L6. The monthly mean exposure concentrations for the 2-year ozone, 2-year ozone/NNK, and the lifetime studies are presented in Figures L7-L18.

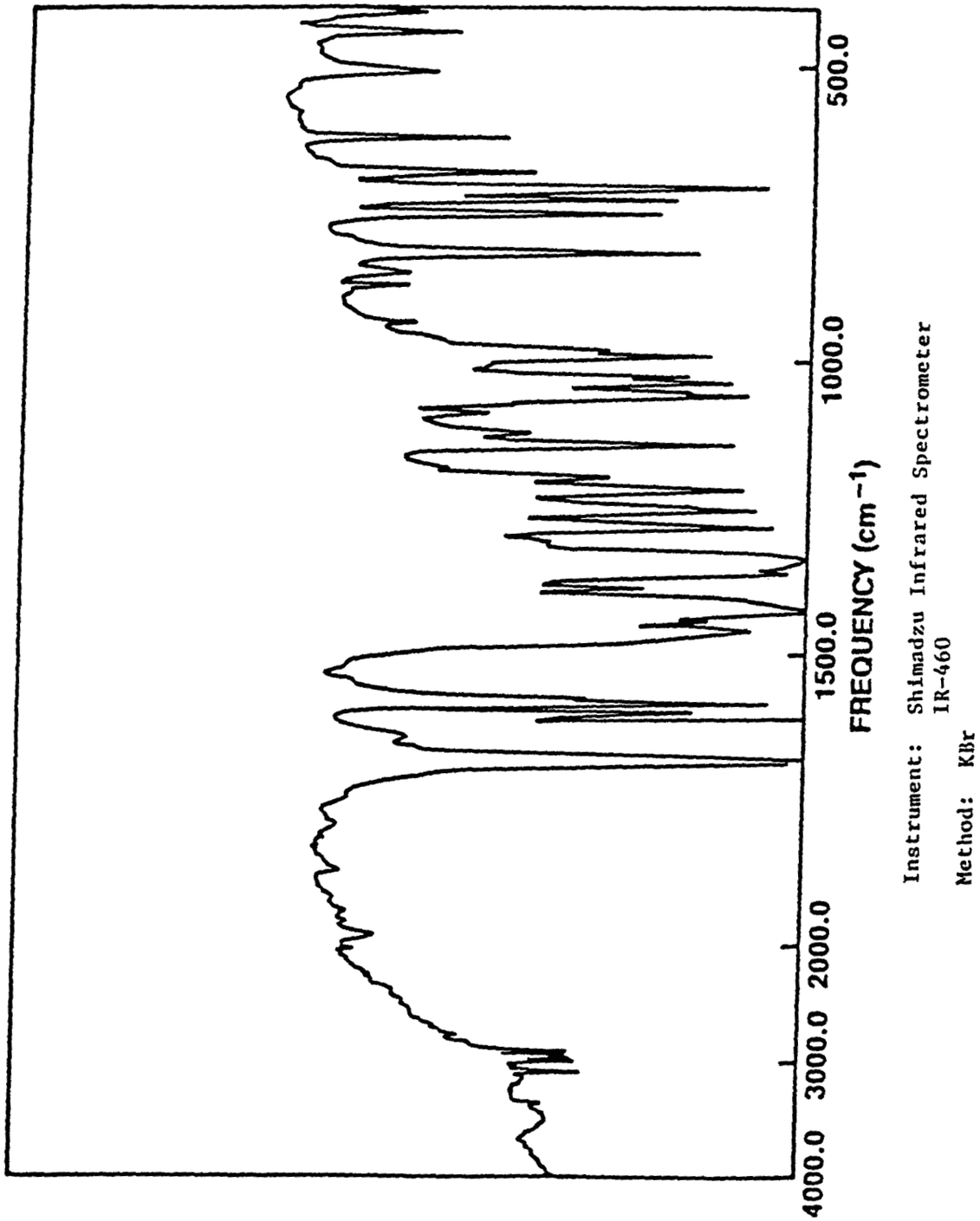


FIGURE L1
Infrared Absorption Spectrum of NNK

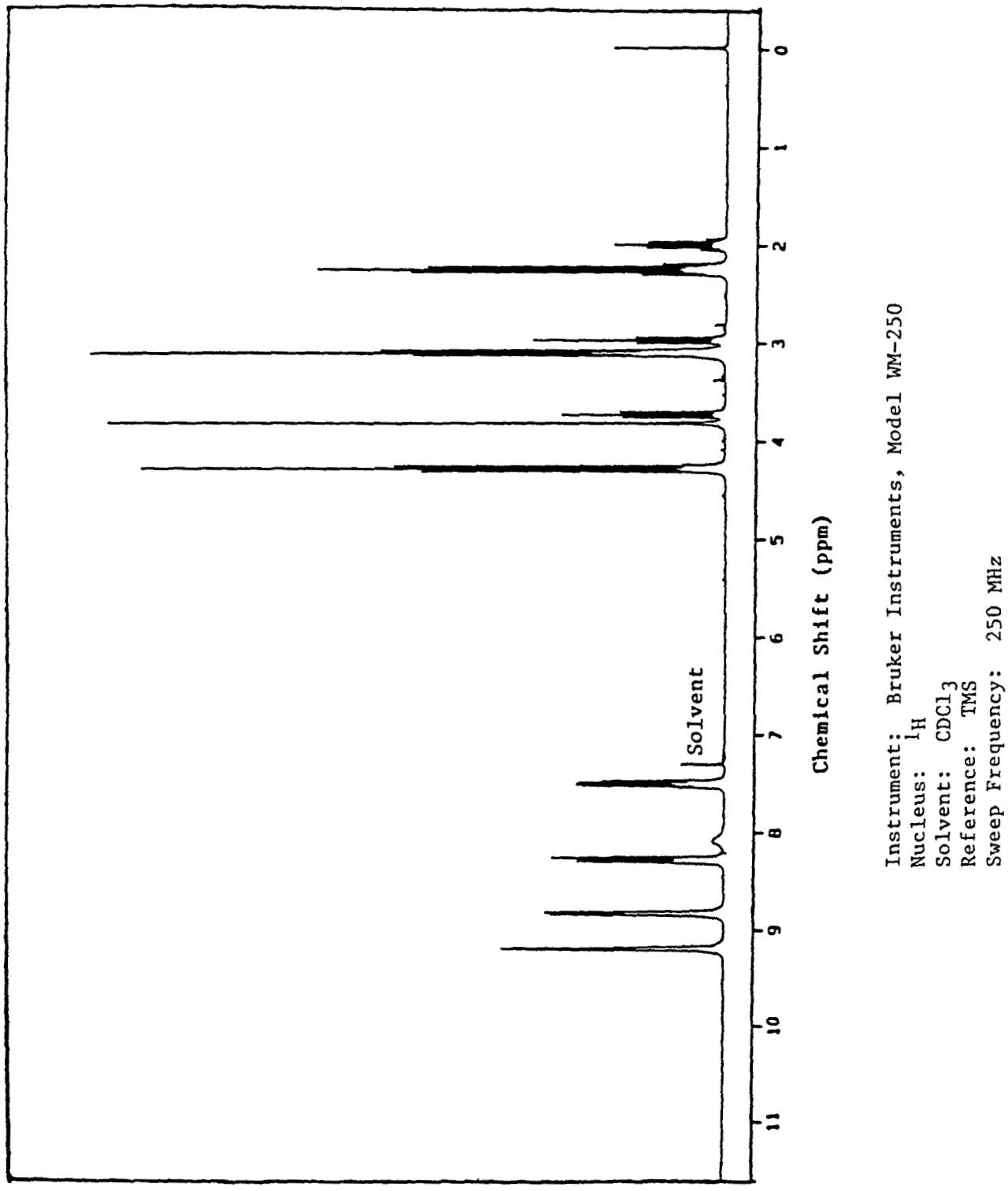


FIGURE L2
Nuclear Magnetic Resonance Spectrum of NNK

TABLE L1
Preparation and Storage of Dose Formulations in the Inhalation Studies of Ozone

4-Week Studies	2-Year Ozone Studies	2-Year Ozone/NNK Study	Lifetime Ozone Studies
Preparation			
Ozone gas was generated by the study lab from >99.9% pure oxygen.	Same as 4-week studies	**Ozone** Same as 4-week studies **NNK/Trioctanoin** NNK/trioctanoin was administered by subcutaneous injection using a semi-automatic syringe that was calibrated at the time of dosing. Dosing solutions were prepared at 0.00, 0.10 and 1.00 mg NNK/mL of trioctanoin. Only solutions that were within 10% of the specified target concentration were used for animal dosing. Dose formulations, except for the initial and final dosing solutions, were prepared every three weeks.	Same as 4-week study
Chemical Lot Number			
Ozone was generated by the study lab from >99.9% pure oxygen and assigned lot number 12636-11.	**Oxygen** 12636-11 12636-58 12733-38 12733-81 12733-115 12733-121 12733-142 12821-7	**Oxygen** 12636-11 12636-58 12733-38 12733-81 12733-115 12733-121 12733-142 12821-7 **NNK** 86-034-01-06 **Trioctanoin** M061289	**Oxygen** 12821-24 12636-58 12733-38 12733-81 12733-115 12733-121 12733-142 12821-7
Maximum Storage Time			
Ozone was generated as needed.	Same as 4-week studies	**Ozone** Same as 4-week studies **NNK/trioctanoin** 3 weeks	Same as 4-week studies

TABLE L1
Preparation and Storage of Dose Formulations in the Inhalation Studies of Ozone (continued)

4-Week Studies	2-Year Ozone Studies	2-Year Ozone/NNK Study	Lifetime Ozone Studies
Storage Conditions			
Cylinders of oxygen were stored at ambient temperatures in the outdoor storage area for compressed gases.	Same as 4-week studies	**Ozone** Same as 4-week studies **NNK/Trioctanoin** 25° C	Same as 4-week studies
Study Laboratory			
Battelle Northwest Laboratories (Richland, WA)	Battelle Northwest Laboratories (Richland, WA)	Battelle Northwest Laboratories (Richland, WA)	Battelle Northwest Laboratories (Richland, WA)

TABLE L2
Results of Analysis of NNK Dose Formulations Administered to Male Rats in the 2-Year Ozone/NNK Study[a]

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[b] (mg/mL)	% Difference from Target
13 November 1989	14,15 November 1989	0.1[c]	0.08	–20
		1.0	0.99	–1
	1 December 1989	1.0[d]	1.04	+4
17 November 1989	17,18 November 1989	0.1	0.10	0
	1 December 1989	0.1[d]	0.10	0
29 November 1989	30 November 1989	0.1	0.12	+20
		1.0	1.04	+4
	6 December 1989	0.1	0.10	+0
	20,21 December 1989	0.1[d]	0.10	+0
		1.0[d]	1.00	+0
29 January 1990	30 January 1990	0.1	0.10	+0
		1.0	1.03	+3
	22-25 February 1990	0.1[d]	0.10	+0
		1.0[d]	1.03	+3
2 April 1990	3 April 1990	0.1	0.11	+10
		1.0	1.01	+1
	13 April 1990	0.1[d]	0.11	+10
		1.0[d]	0.98	–2

[a] Dosing volume is equal to 1 mL/kg body weight
[b] Results of duplicate analyses
[c] Dose formulation not used
[d] Animal room sample

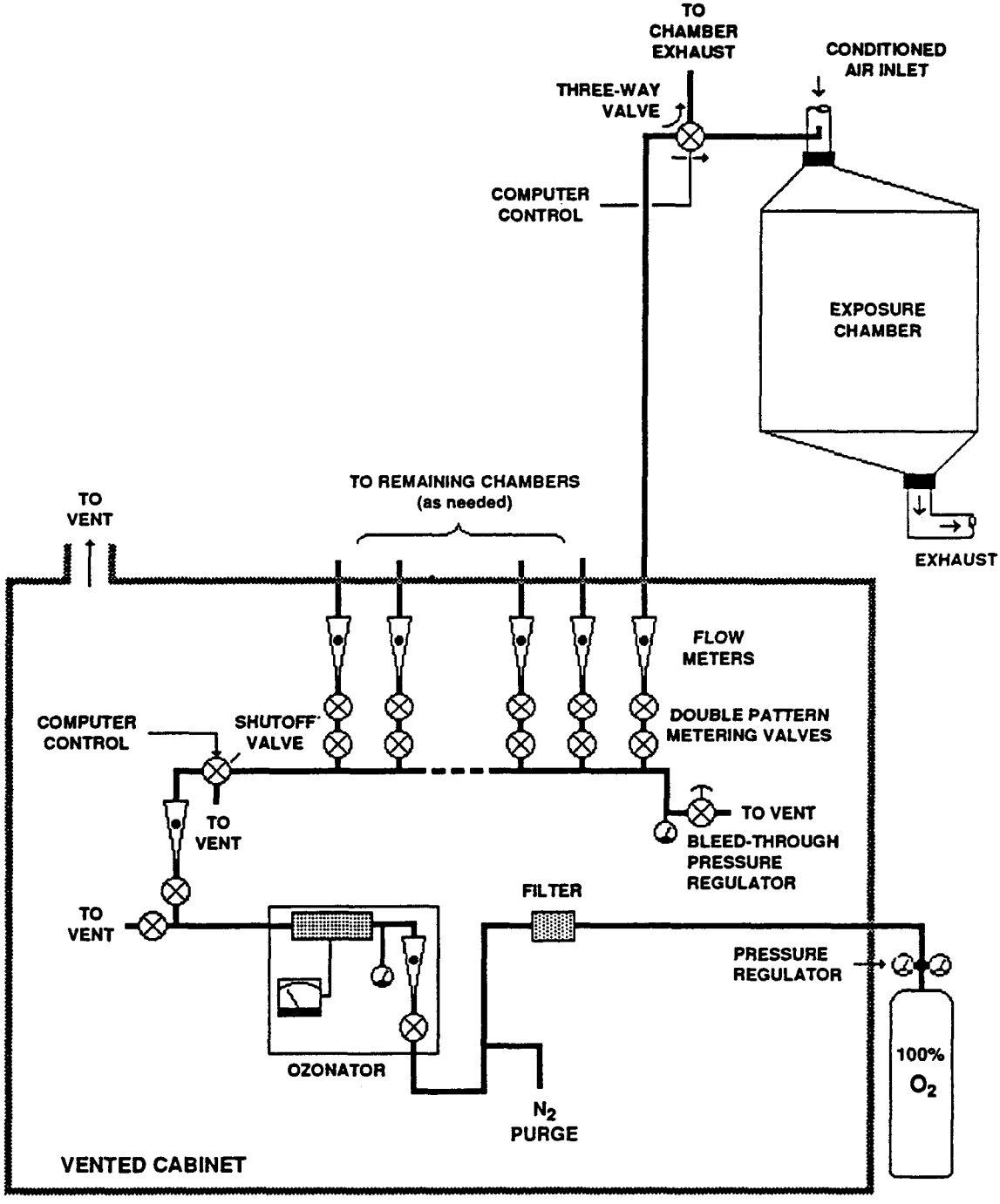
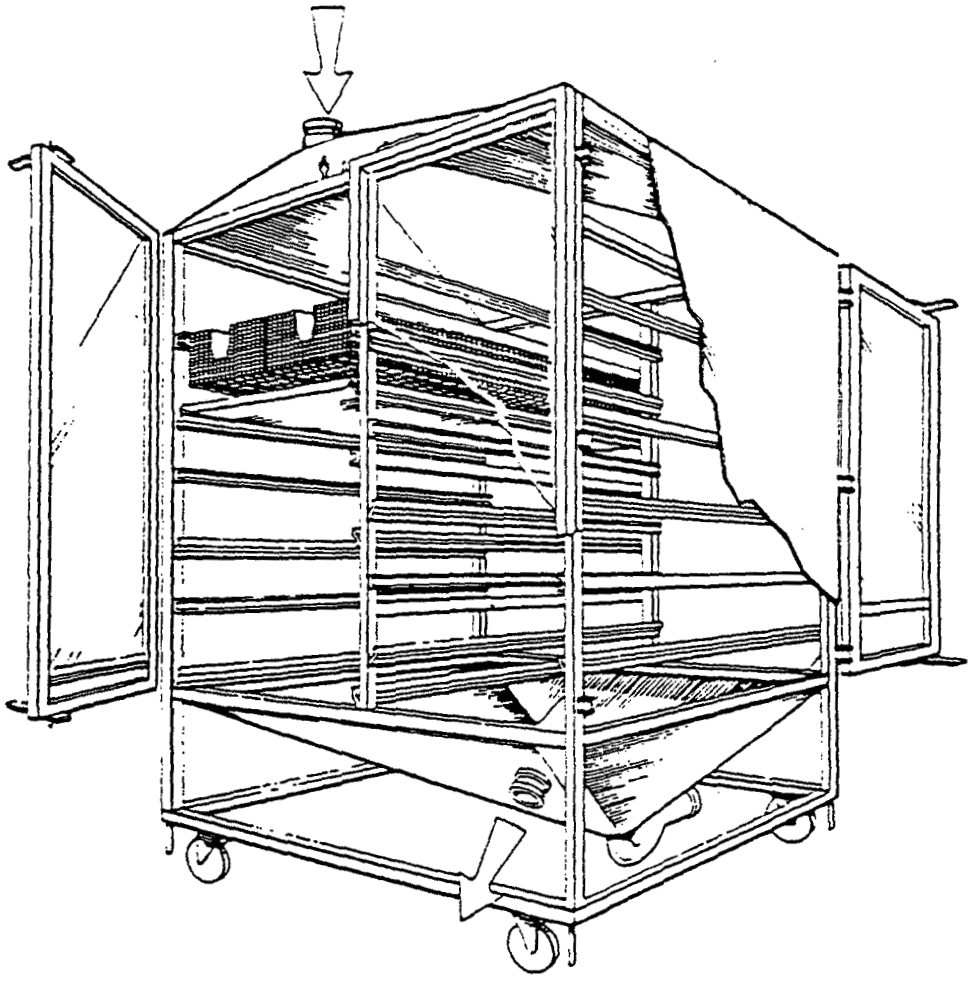


FIGURE L3
Ozone Vapor Generation and Delivery System



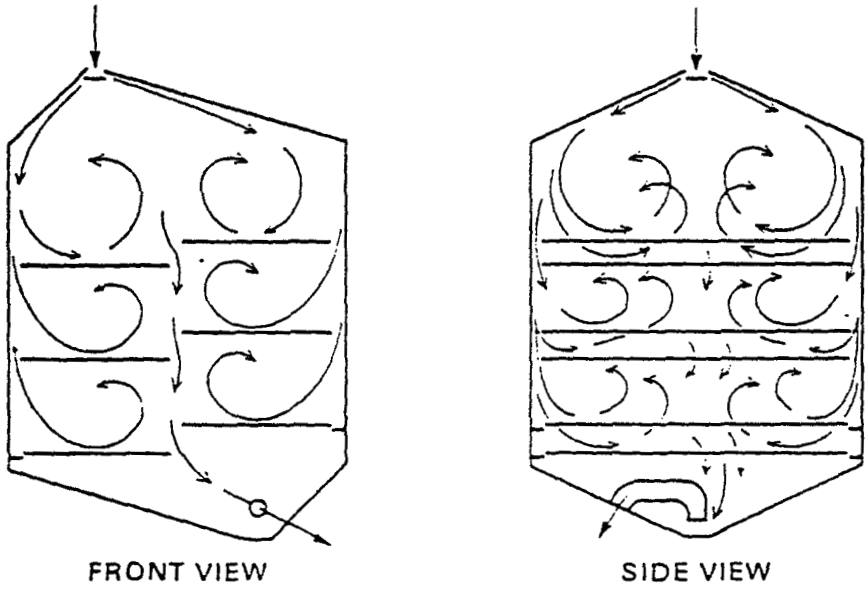


FIGURE L4
Ozone Inhalation Exposure Chamber

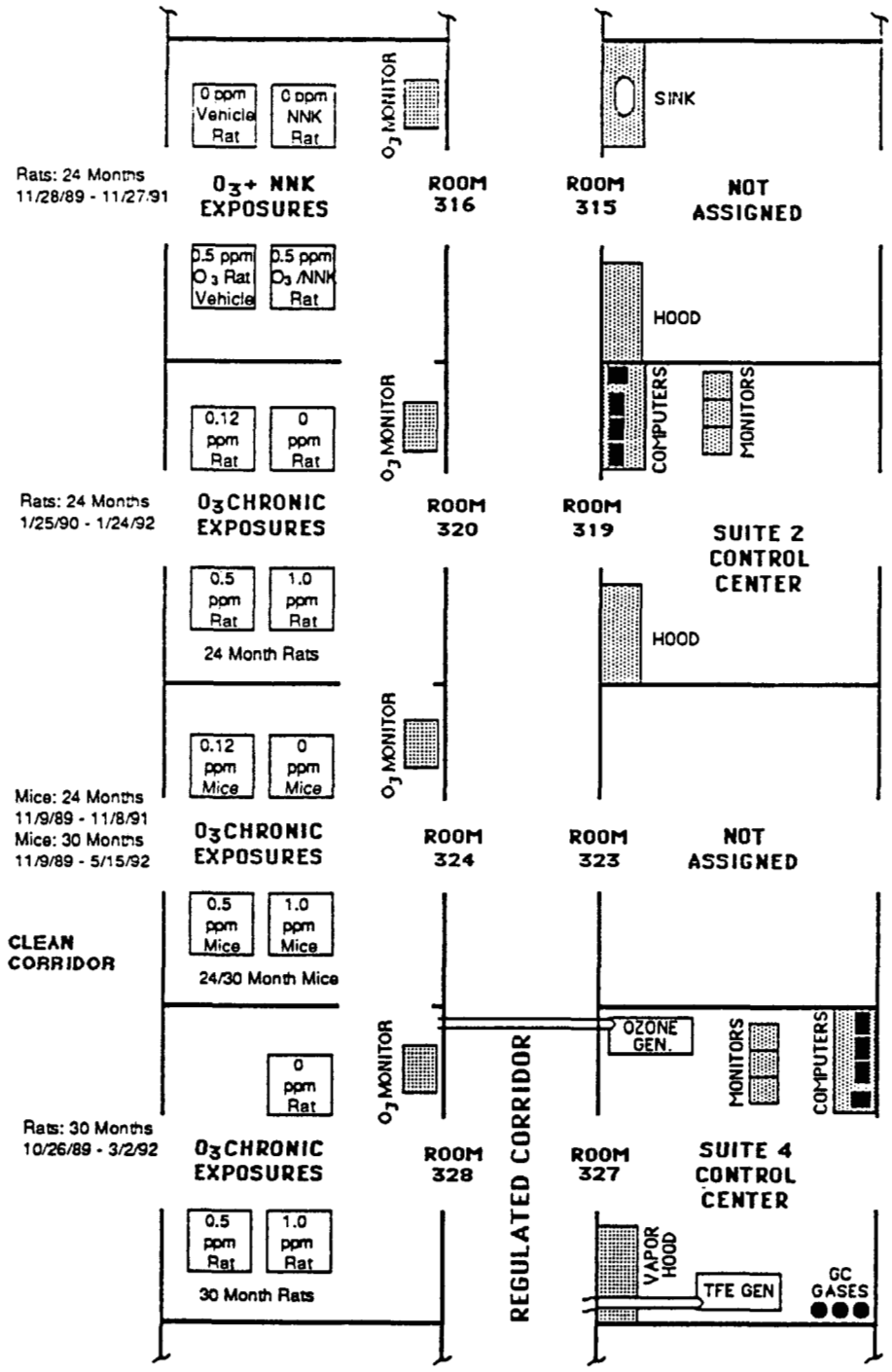


FIGURE L5
Ozone Exposure Suite

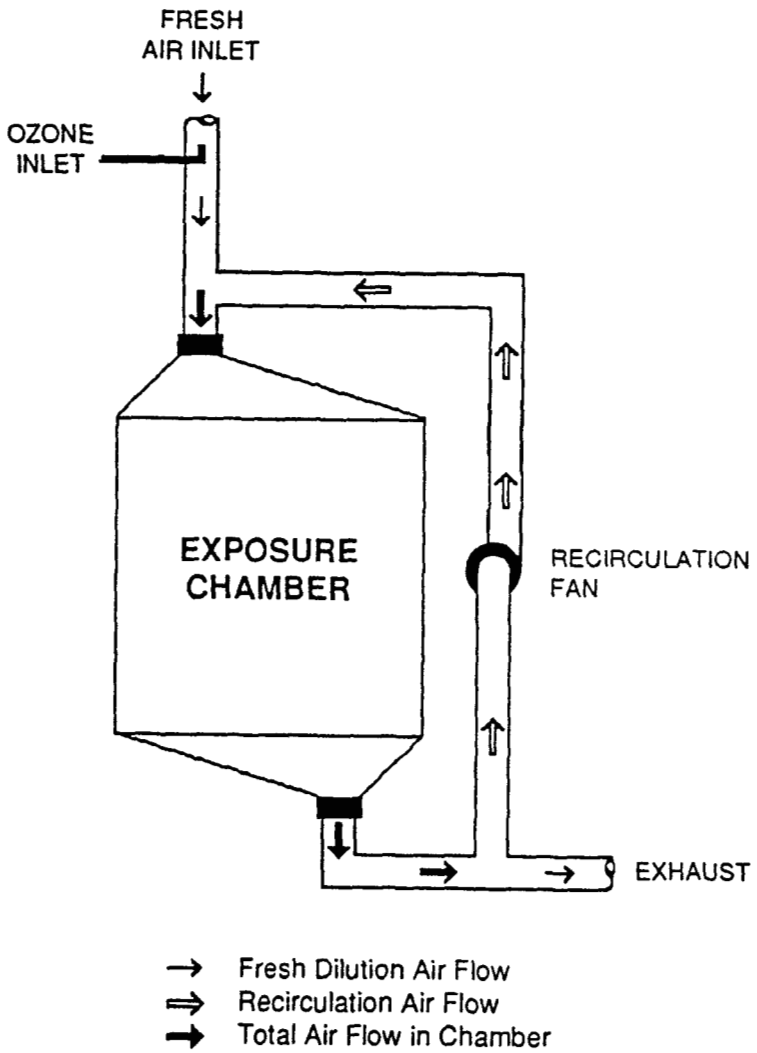


FIGURE 1.6
Ozone Inhalation Exposure Chamber Recirculation System

TABLE L3
Summary of Chamber Concentrations in the 4-Week Inhalation Studies of Ozone

Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers		
0.5	199	0.529 ± 0.073
1.0	200	1.070 ± 0.155
Mouse Chambers		
0.5	199	0.529 ± 0.073
1.0	200	1.070 ± 0.155

[a] Mean ± standard deviation

TABLE L4
Summary of Chamber Concentrations in the 2-Year Inhalation Studies of Ozone

Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers		
0.12	3,904	0.120 ± 0.006
0.5	3,838	0.501 ± 0.023
1.0	3,831	0.998 ± 0.040
Mouse Chambers		
0.12	3,940	0.121 ± 0.007
0.5	3,883	0.506 ± 0.029
1.0	3,885	1.02 ± 0.065

[a] Mean ± standard deviation

TABLE L5
Summary of Chamber Concentrations in the 2-Year Inhalation Study of Ozone/NNK

Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
0.5 Ozone	3,975	0.498 ± 0.022
0.5 Ozone (V)[b]	3,983	0.495 ± 0.022

[a] Mean ± standard deviation
[b] Trioctanoin vehicle control

TABLE L6
Summary of Chamber Concentrations in the Lifetime Inhalation Studies of Ozone

Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers		
0.5	4,563	0.497 ± 0.020
1.0	4,569	1.01 ± 0.042
Mouse Chambers		
0.5	4,792	0.504 ± 0.028
1.0	4,788	1.01 ± 0.061

[a] Mean ± standard deviation

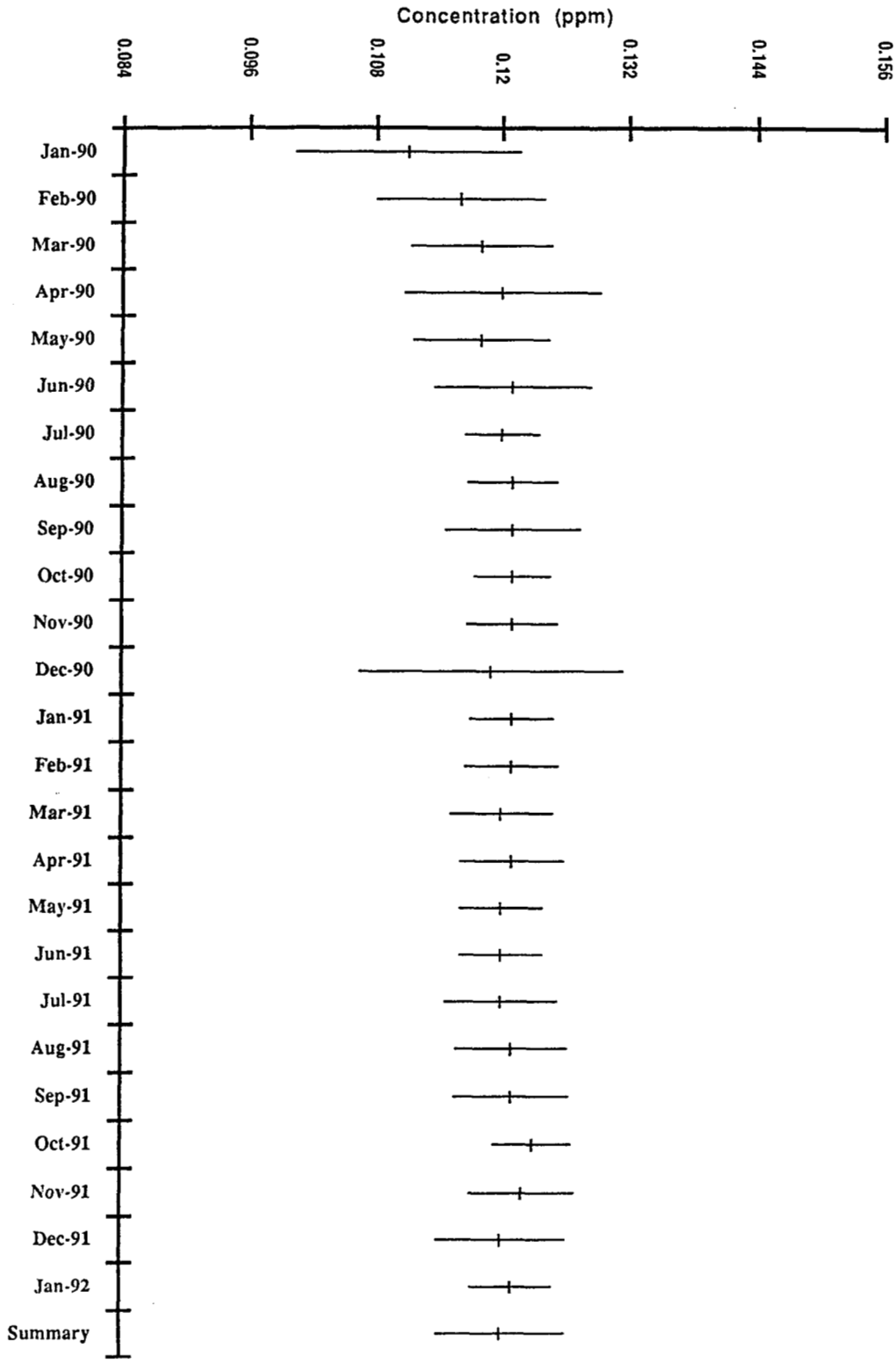


FIGURE L7
Monthly Mean Concentration and Standard Deviation in the 0.12 ppm Ozone Rat Exposure Chamber for the 2-Year Study

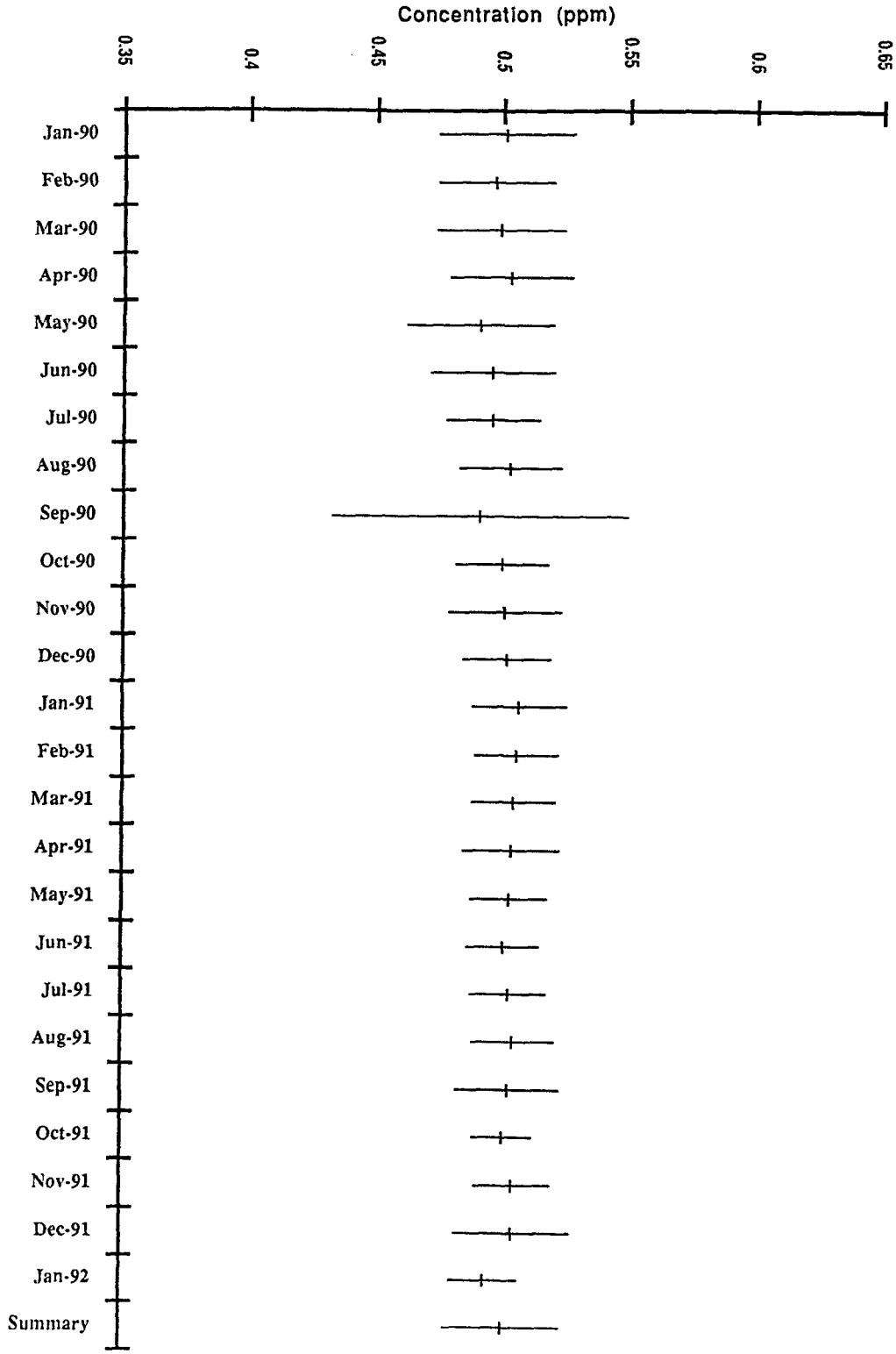


FIGURE L8
Monthly Mean Concentration and Standard Deviation in the 0.5 ppm Ozone Rat Exposure Chamber for the 2-Year Study

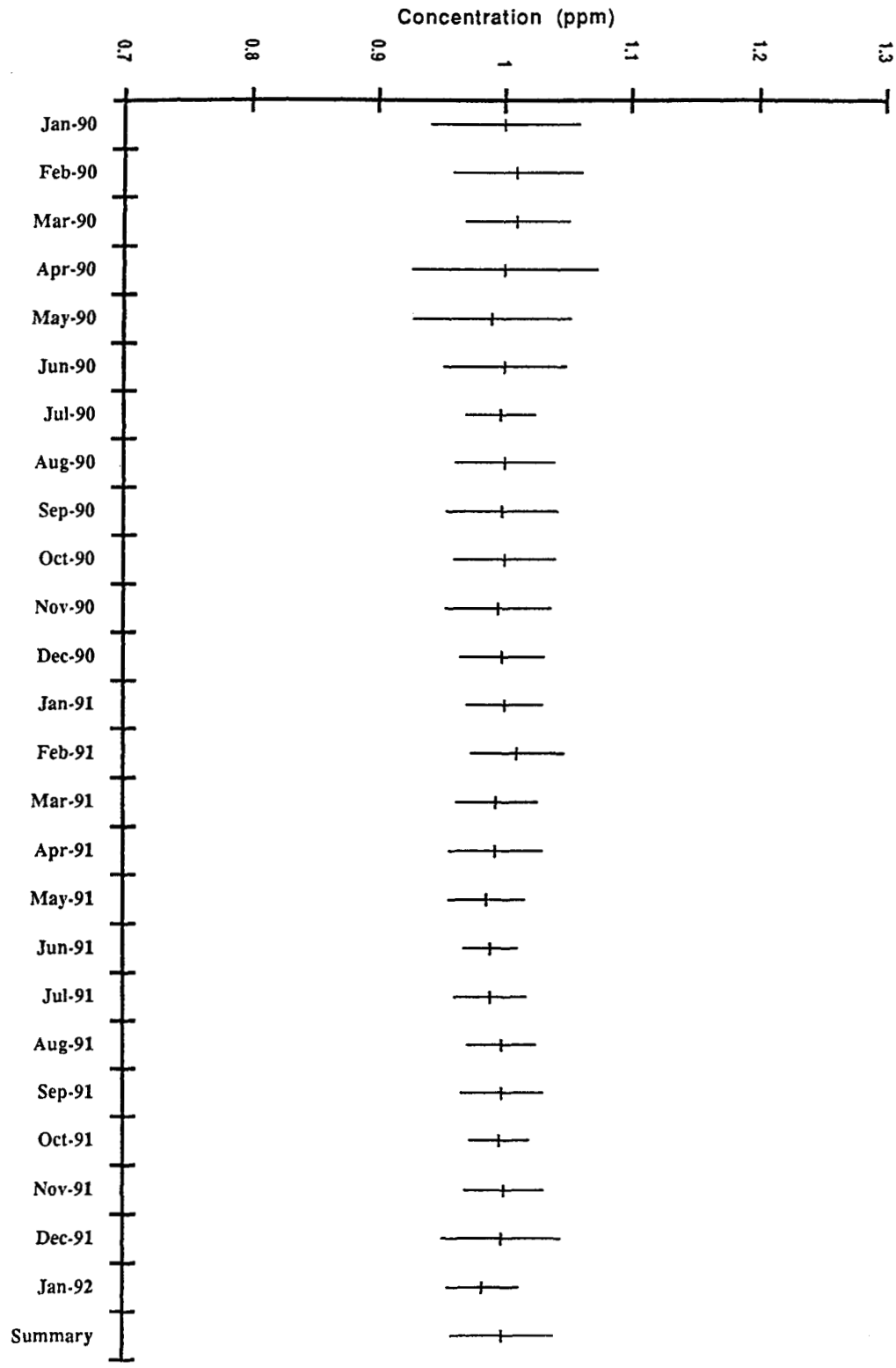


FIGURE L9
Monthly Mean Concentration and Standard Deviation in the 1.0 ppm Ozone Rat Exposure Chamber for the 2-Year Study

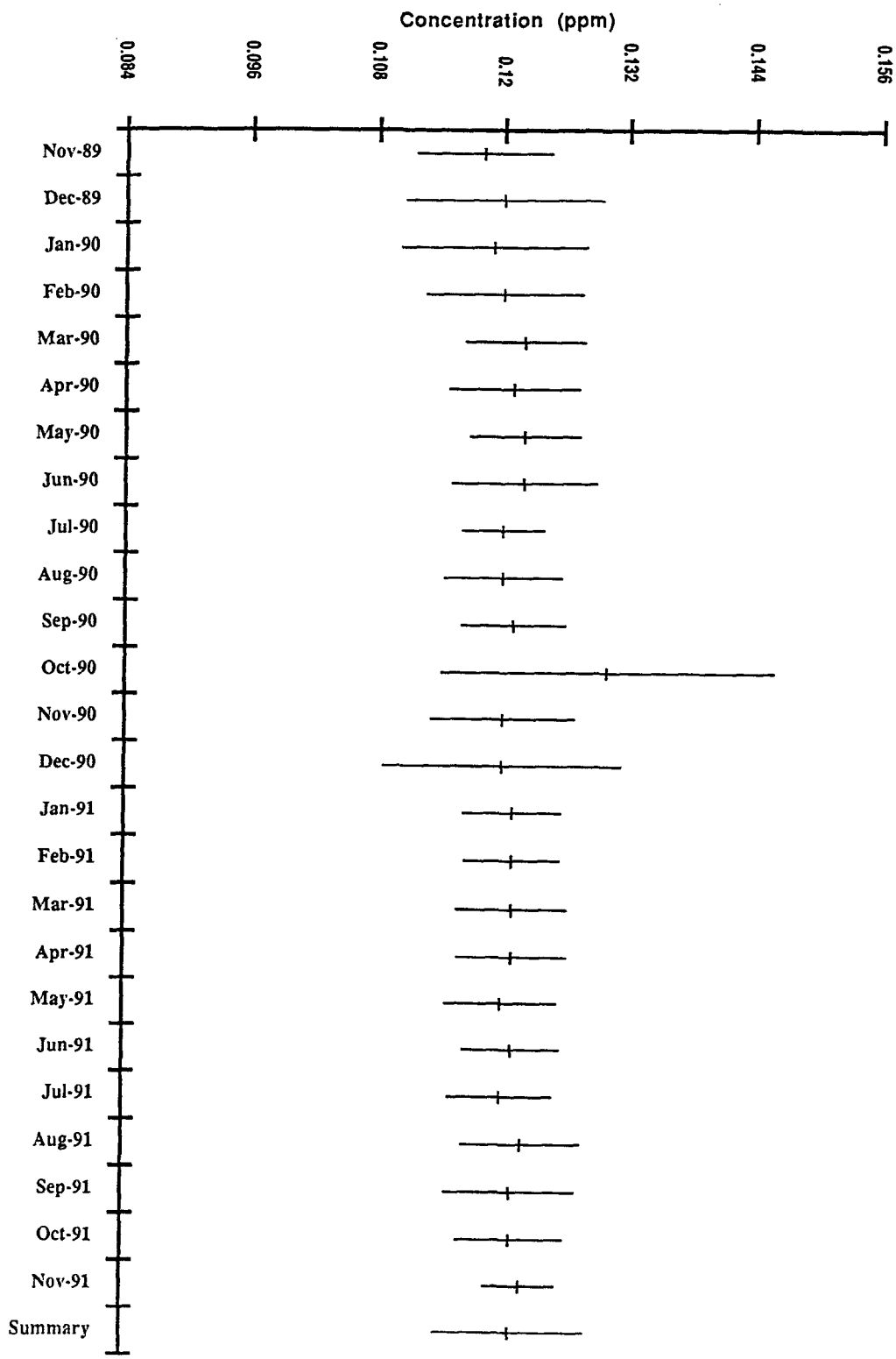


FIGURE L10
Monthly Mean Concentration and Standard Deviation in the 0.12 ppm Ozone Mouse Exposure Chamber for the 2-Year Study

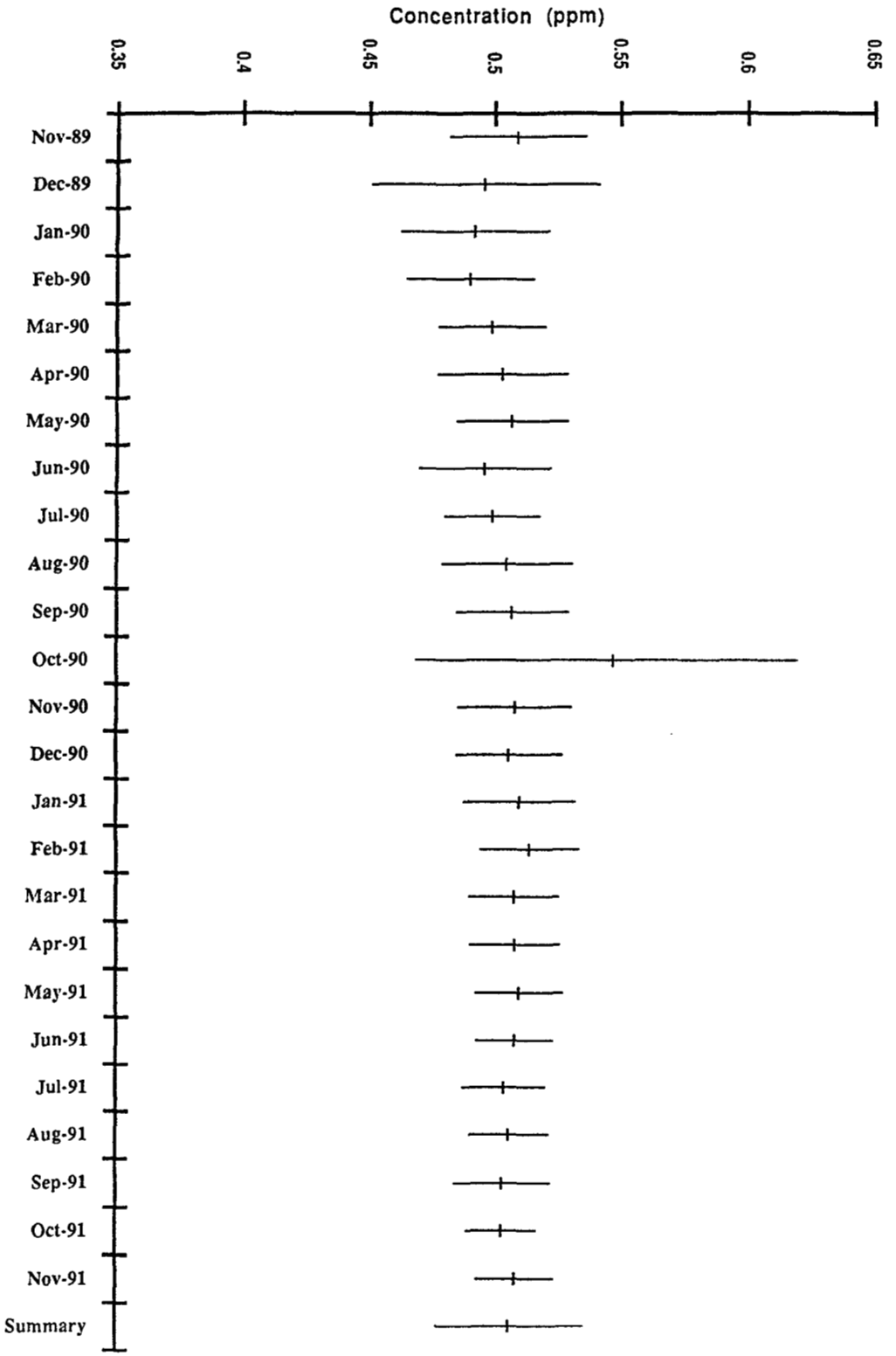


FIGURE L11
Monthly Mean Concentration and Standard Deviation in the 0.5 ppm Ozone Mouse Exposure Chamber for the 2-Year Study

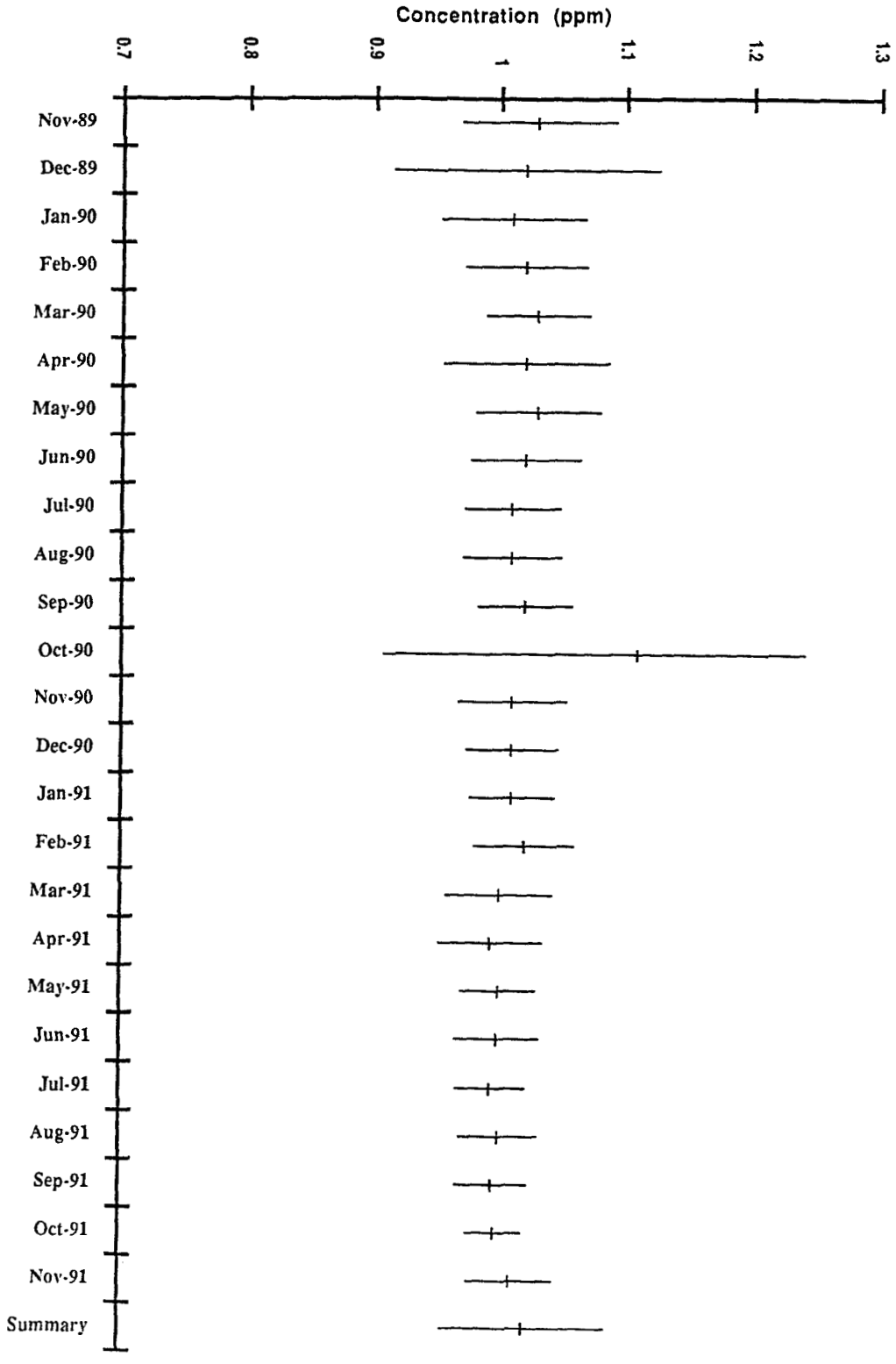


FIGURE L12
Monthly Mean Concentration and Standard Deviation in the 1.0 ppm Ozone Mouse Exposure Chamber for the 2-Year Study

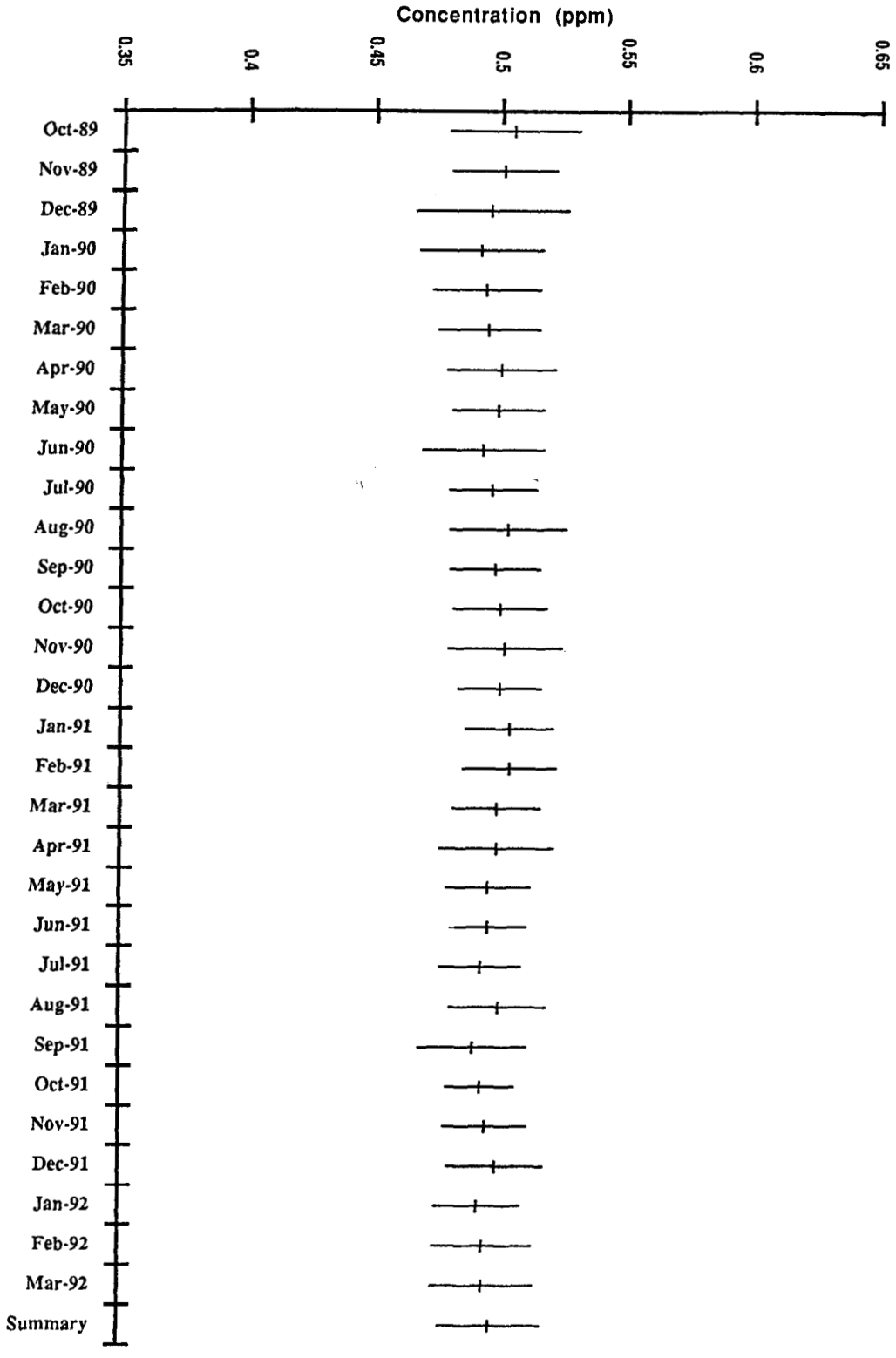


FIGURE L13
Monthly Mean Concentration and Standard Deviation in the 0.5 ppm Ozone Rat Exposure Chamber for the Lifetime Study

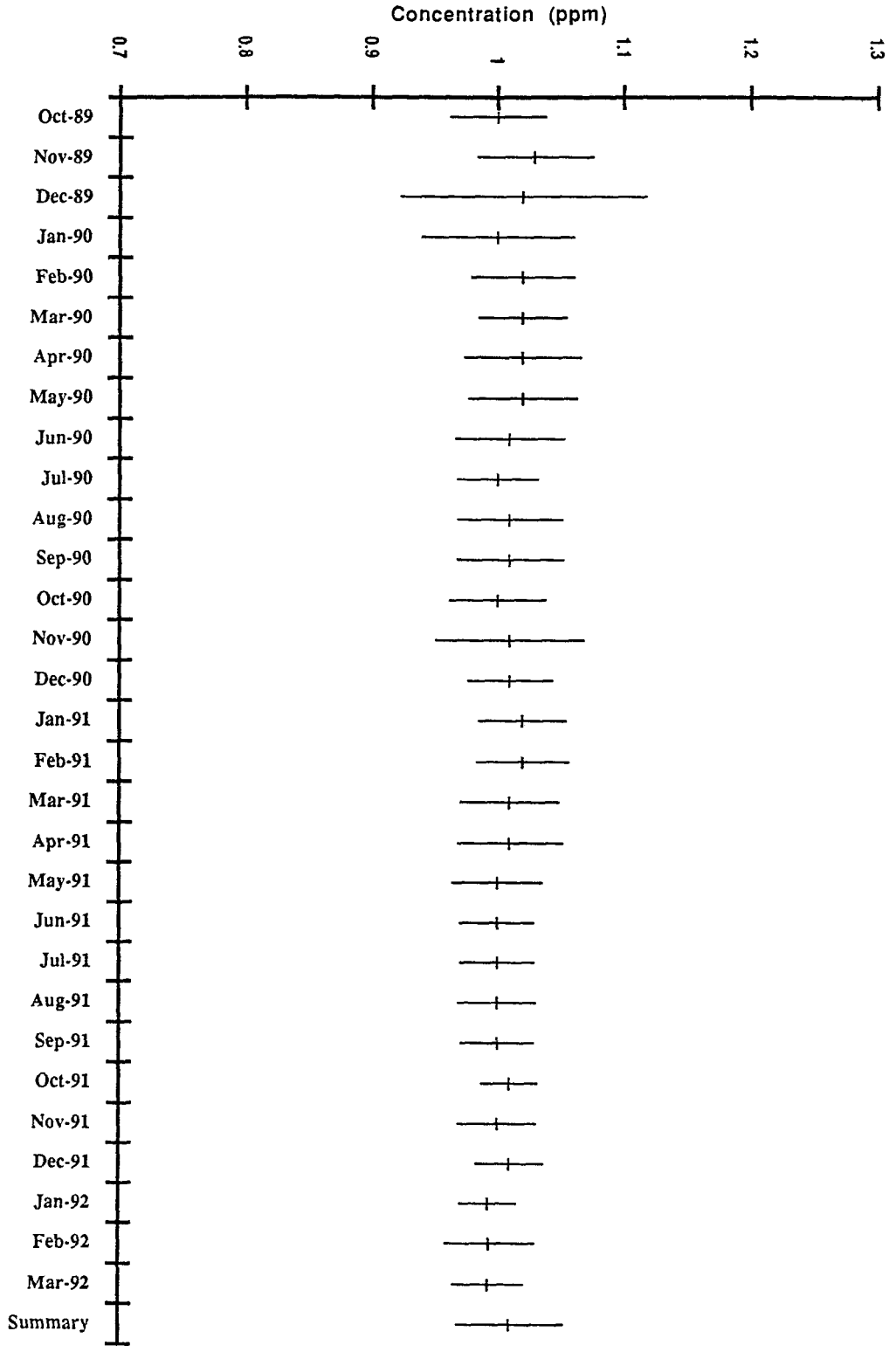


FIGURE L14
Monthly Mean Concentration and Standard Deviation in the 1.0 ppm Ozone Rat Exposure Chamber for the Lifetime Study

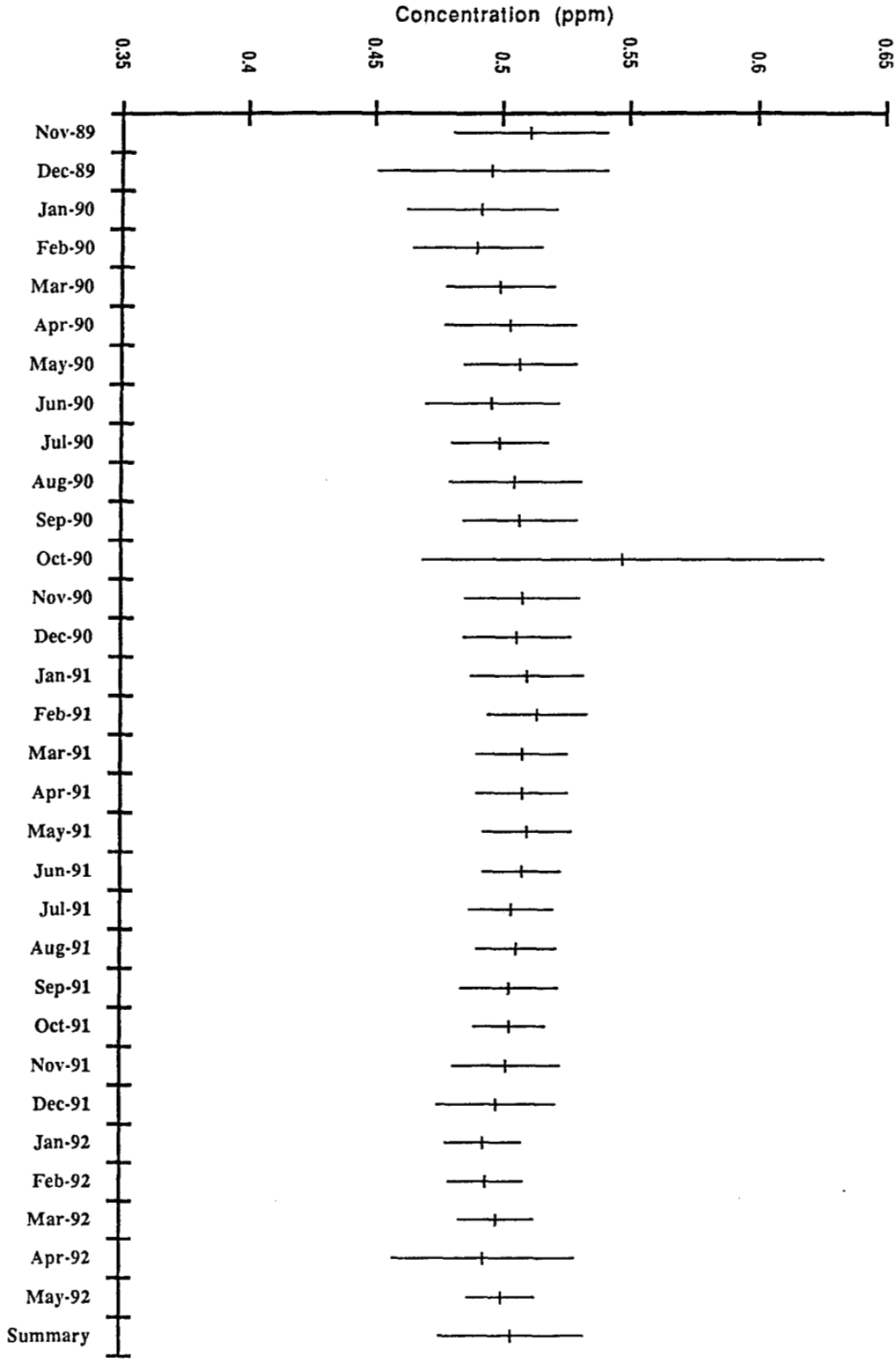


FIGURE L15
Monthly Mean Concentration and Standard Deviation in the 0.5 ppm Ozone Mouse Exposure Chamber for the Lifetime Study

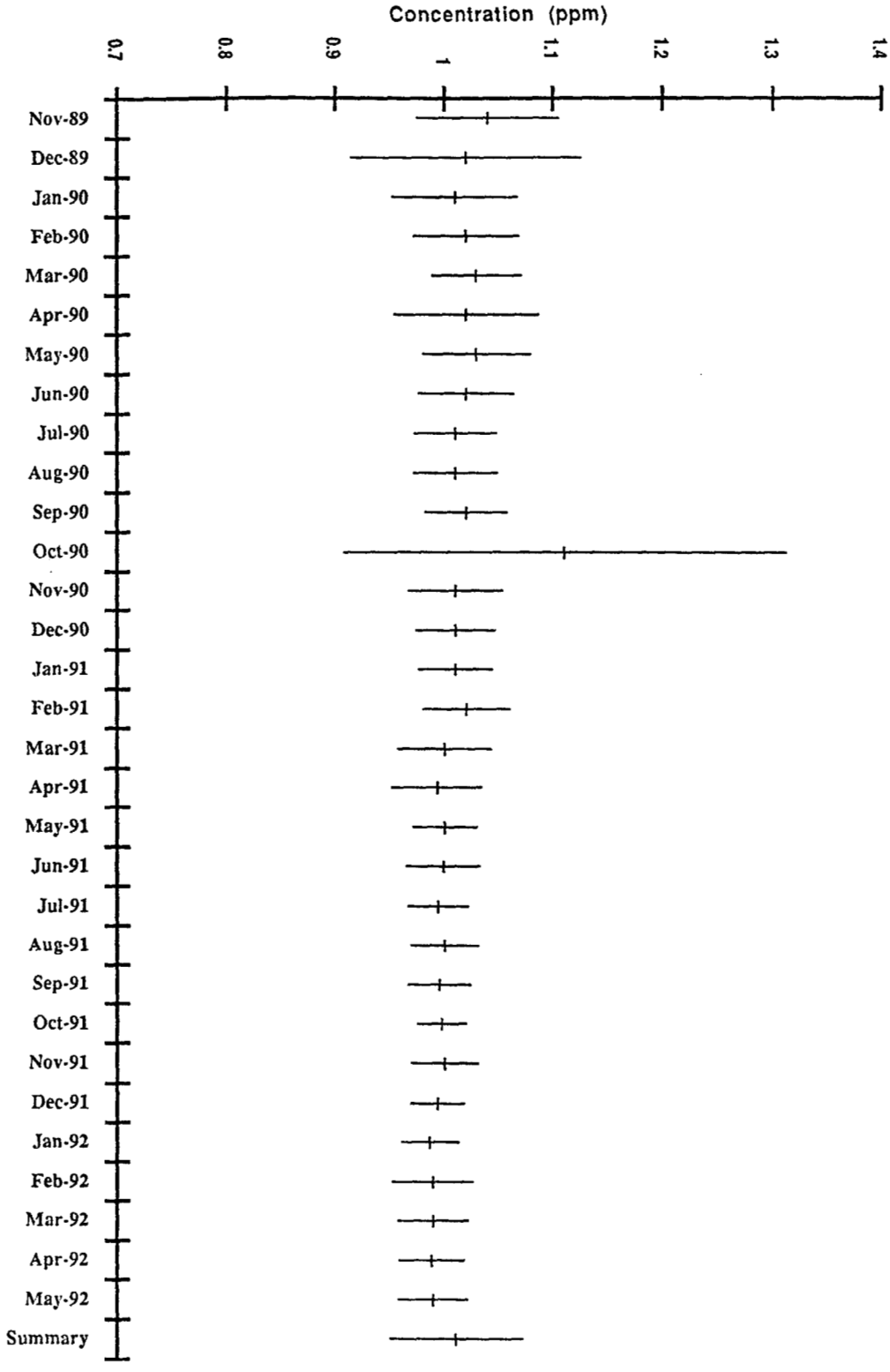


FIGURE L16
Monthly Mean Concentration and Standard Deviation in the 1.0 ppm Ozone Mouse Exposure Chamber for the Lifetime Study

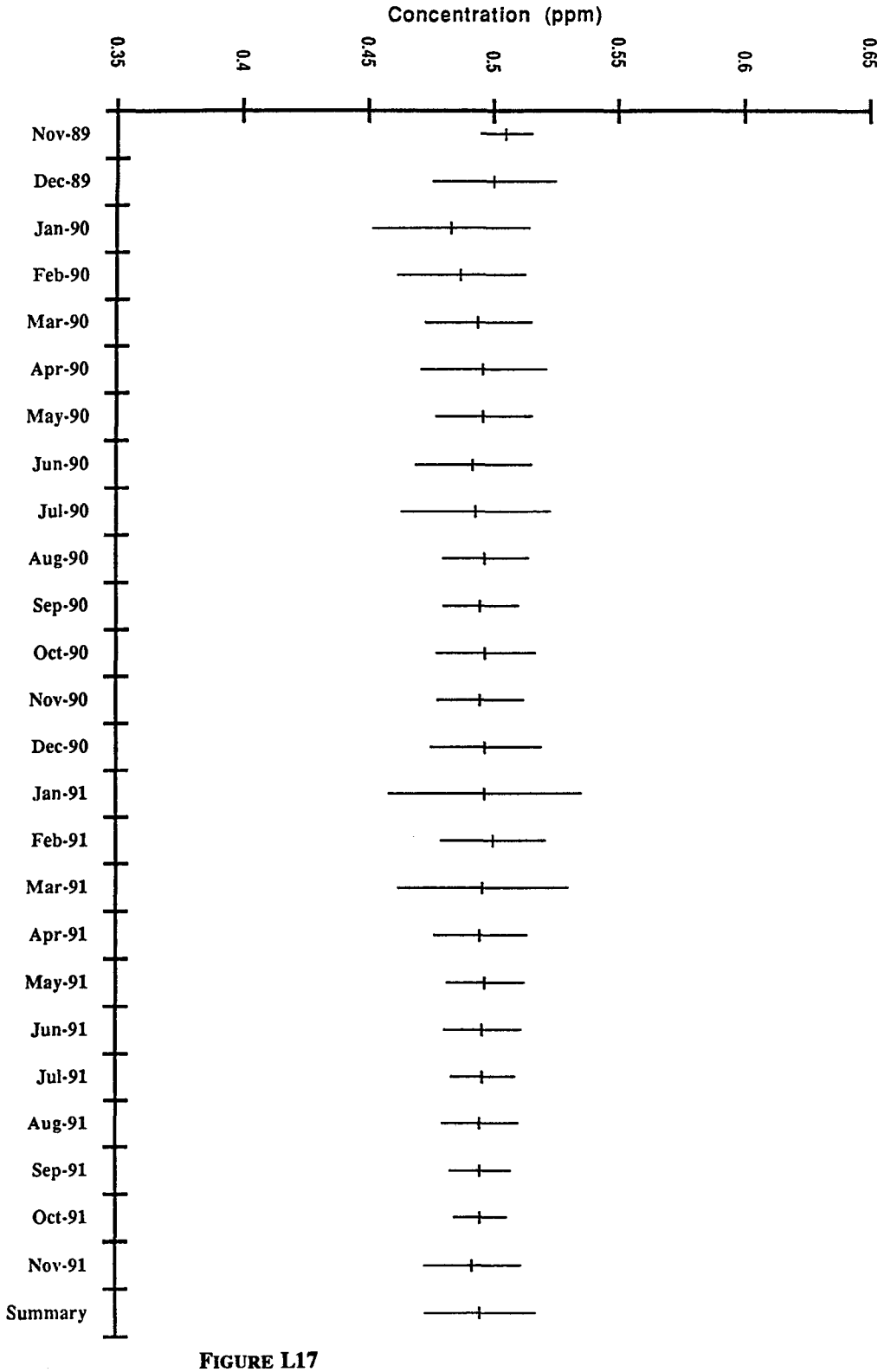


FIGURE L17
Monthly Mean Concentration and Standard Deviation in the 0.5 ppm (Vehicle) Ozone/NNK Rat Exposure Chamber for the 2-Year Study

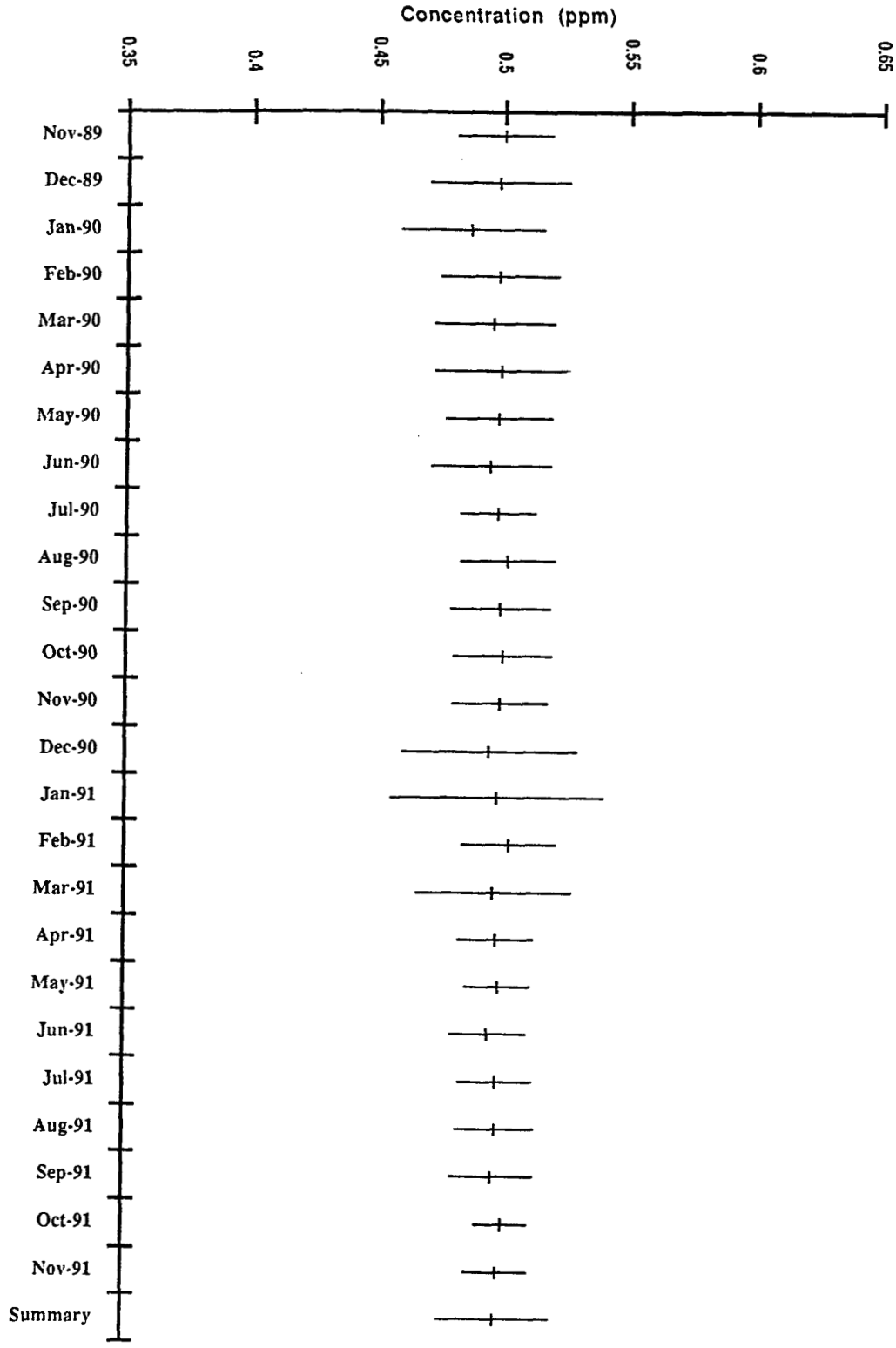


FIGURE L18
Monthly Mean Concentration and Standard Deviation in the 0.5 ppm Ozone/NNK Rat Exposure Chamber for the 2-Year Study

APPENDIX M
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE M1 Ingredients of NIH-07 Rat and Mouse Ration 300
TABLE M2 Vitamins and Minerals in NIH-07 Rat and Mouse Ration 300
TABLE M3 Nutrient Composition of NIH-07 Rat and Mouse Ration 301
TABLE M4 Contaminant Levels in NIH-07 Rat and Mouse Ration 302

TABLE M1
Ingredients of NIH-07 Rat and Mouse Ration[a]

Ingredients[b]	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

[a] NCI, 1976; NIH, 1978
[b] Ingredients were ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE M2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 μg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

TABLE M3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	23.43 ± 0.54	22.20 – 24.30	27
Crude Fat (% by weight)	5.29 ± 0.16	5.00 – 5.60	27
Crude Fiber (% by weight)	3.51 ± 0.41	2.60 – 4.30	27
Ash (% by weight)	6.37 ± 0.18	6.11 – 6.81	27
Amino Acids (% of total diet)			
Arginine	1.287 ± 0.084	1.100 – 1.390	10
Cystine	0.306 ± 0.075	0.181 – 0.400	10
Glycine	1.160 ± 0.050	1.060 – 1.220	10
Histidine	0.580 ± 0.024	0.531 – 0.608	10
Isoleucine	0.917 ± 0.034	0.867 – 0.965	10
Leucine	1.972 ± 0.052	1.850 – 2.040	10
Lysine	1.273 ± 0.051	1.200 – 1.370	10
Methionine	0.437 ± 0.115	0.306 – 0.699	10
Phenylalanine	0.994 ± 0.125	0.665 – 1.110	10
Threonine	0.896 ± 0.055	0.824 – 0.985	10
Tryptophan	0.223 ± 0.160	0.107 – 0.671	10
Tyrosine	0.677 ± 0.105	0.564 – 0.794	10
Valine	1.089 ± 0.057	0.962 – 1.170	10
Essential Fatty Acids (% of total diet)			
Linoleic	2.389 ± 0.233	1.830 – 2.570	9
Linolenic	0.277 ± 0.036	0.210 – 0.320	9
Vitamins			
Vitamin A (IU/kg)	6,520 ± 1,510	4,180 – 11,450	27
Vitamin D (IU/kg)	4,450 ± 1,382	3,000 – 6,300	4
α-Tocopherol (ppm)	36.92 ± 9.32	22.5 – 48.9	9
Thiamine (ppm)	18.18 ± 1.52	15.0 – 21.0	27
Riboflavin (ppm)	7.92 ± 0.93	6.10 – 9.00	10
Niacin (ppm)	100.95 ± 25.92	65.0 – 150.0	9
Pantothenic Acid (ppm)	30.30 ± 3.60	23.0 – 34.6	10
Pyridoxine (ppm)	9.25 ± 2.62	5.60 – 14.0	10
Folic acid (ppm)	2.51 ± 0.64	1.80 – 3.70	10
Biotin (ppm)	0.267 ± 0.049	0.19 – 0.35	10
Vitamin B_{12} (ppb)	40.14 ± 20.04	10.6 – 65.0	10
Choline (ppm)	3,068 ± 314	2,400 – 3,430	9
Minerals			
Calcium (%)	1.17 ± 0.09	1.00 – 1.49	27
Phosphorus (%)	0.93 ± 0.04	0.85 – 1.00	27
Potassium (%)	0.887 ± 0.067	0.772 – 0.971	8
Chloride (%)	0.526 ± 0.092	0.380 – 0.635	8
Sodium (%)	0.315 ± 0.344	0.258 – 0.370	10
Magnesium (%)	0.168 ± 0.008	0.151 – 0.180	10
Sulfur (%)	0.274 ± 0.063	0.208 – 0.420	10
Iron (ppm)	356.2 ± 90.0	255.0 – 523.0	10
Manganese (ppm)	92.24 ± 5.35	81.70 – 99.40	10
Zinc (ppm)	58.14 ± 9.91	46.10 – 81.60	10
Copper (ppm)	11.50 ± 2.40	8.090 – 15.39	10
Iodine (ppm)	3.70 ± 1.14	1.52 – 5.83	10
Chromium (ppm)	1.71 ± 0.45	0.85 – 2.09	9
Cobalt (ppm)	0.797 ± 0.23	0.490 – 1.150	6

TABLE M4
Contaminant Levels in NIH-07 Rat and Mouse Ration

	Mean ± Standard Deviation[a]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.36 ± 0.18	0.10 – 0.70	25
Cadmium (ppm)	<0.20		25
Lead (ppm)	0.30 ± 0.23	0.10 – 1.30	25
Mercury (ppm)	<0.05		25
Selenium (ppm)	0.33 ± 0.13	0.05 – 0.60	25
Aflatoxins (ppb)[b]	<5.00		25
Nitrate nitrogen (ppm)	12.24 ± 5.18	2.90 – 21.0	25
Nitrite nitrogen (ppm)	0.22 ± 0.18	<0.10 – 0.70	25
BHA (ppm)	1.81 ± 1.58	<1.00 – 10.0	25
BHT (ppm)	1.55 ± 1.53	<1.00 – 8.00	25
Aerobic plate count (CFU/g)[c]	73,867 ± 138,519	4,100 – 710,000	25
Coliform (MPN/g)[d]	3.04 ± 0.19	3.00 – 4.00	25
E. coli (MPN/g)[d]	<3.00		25
Total Nitrosoamines (ppb)[e]	7.74 ± 2.42	4.80 – 16.50	25
N-Nitrosodimethylamine (ppb)[e]	5.88 ± 1.88	3.80 – 13.00	25
N-Nitrosopyrrolidine (ppb)[e]	1.86 ± 1.05	1.00 – 4.30	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.28 ± 0.26	<0.05 – 1.00	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

[a] For values less than the limit of detection, the detection limit is given as the mean.
[b] No aflatoxin measurement was recorded for the lot milled 10-02-89.
[c] CFU = colony forming units.
[d] MPN = most probable number.
[e] All values were corrected for percent recovery.

APPENDIX N
SENTINEL ANIMAL PROGRAM

METHODS 304
TABLE N1 Murine Virus Antibody Determinations for Rats and Mice in the 4-Week, 2-Year, and Lifetime Inhalation Studies of Ozone and Ozone/NNK 307

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 as many as 16 randomly selected rats and mice during the 4-week, 2-year, and lifetime studies. Blood from each animal was collected, allowed to clot, and the serum separated. The samples were processed appropriately and sent to Microbiological Associates, Inc. (Bethesda, MD) for determination of antibody titers. The laboratory serolgy 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	
4-Week study	
ELISA	
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/sialodacrydoadenitis virus)	Study termination
Sendai	Study termination
Hemagglutination inhibition	
H-1 (Toolan's H-1 virus)	Study termination
KRV (Kilham rat virus)	Study termination
2-Year study	
ELISA	
Mycoplasma arthritidis	24 months
M. pulmonis	24 months
PVM	6, 12, 18, and 24 months
RCV/SDA	6, 12, 18, and 24 months
Sendai	6, 12, 18, and 24 months
Immunofluorescence assay	
RCV/SDA	18 months
Hemagglutination inhibition	
H-1	6, 12, 18, and 24 months
KRV	6, 12, 18, and 24 months

Method and Test	Time of Analysis
RATS (continued)	
2-Year ozone/NNK study	
ELISA	
M. arthritidis	24 months
M. pulmonis	24 months
PVM	6, 12, 18, and 24 months
RCV/SDA	6, 12, 18, and 24 months
Sendai	6, 12, 18, and 24 months
Immunofluorescence Assay	
Sendai	24 months
Hemagglutination Inhibition	
H-1	6, 12, 18, and 24 months
KRV	6, 12, 18, and 24 months
Lifetime study	
ELISA	
M. arthritidis	30 months
M. pulmonis	30 months
PVM	6, 12, 18, and 30 months
RCV/SDA	6, 12, 18, and 30 months
Sendai	6, 12, 18, and 30 months
Immunofluorescence assay	
PVM	12 months
Hemagglutination inhibition	
H-1	6, 12, 18, and 30 months
KRV	6, 12, 18, and 30 months
MICE	
2-Year study	
ELISA	
Ectromelia virus	6, 12, 18, and 24 months
EDIM (epizootic diarrhea of infant mice)	6, 12, and 24 months
GDVII (mouse encephalomyelitis virus)	6, 12, 18, and 24 months
LCM (lymphocytic choriomeningitis virus)	6, 12, 18, and 24 months
Mouse adenoma virus	6, 1,2 18, and 24 months
MHV (mouse hepatitis virus)	6, 12, 18, and 24 months
M. arthritidis	24 months
M pulmonis	24 months
PVM	6, 12, 18, and 24 months
Reovirus 3	6, 12, 18, and 24 months
Sendai	6, 12, 18, and 24 months

Method and Test	Time of Analysis
MICE (continued)	
2-Year study (continued)	
Immunofluorescence assay	
EDIM	18, and 24 months
MVM (minute virus of mice)	6 months
Reovirus 3	6 and 12 months
Hemagglutination inhibition	
MVM	12, 18, and 24 months
K (papovavirus)	6, 12, 18, and 24 months
Polyoma virus	6, 12, 18, and 24 months
Reovirus 3	6 months
Lifetime study	
ELISA	
Ectromelia virus	6, 12, and 30 months
EDIM	6, 12, and 30 months
GDVII	6, 12, and 30 months
LCM	6, 12, and 30 months
Mouse adenoma virus	6, 12, and 30 months
MHV	6, 12, and 30 months
M. arthritidis	30 months
M. pulmonis	30 months
PVM	6, 12, and 30 months
Reovirus 3	6, 12, and 30 months
Sendai	6, 12, and 30 months
Immunofluorescence assay	
MHV	30 months
MVM	6 months
Reovirus 3	6, 12 months
Hemagglutination inhibition	
MVM	12 and 30 months
K	6, 12, and 30 months
Polyoma virus	6, 12, and 30 months
Reovirus 3	6 months

Results of serology tests are presented in Table N1.

TABLE N1
Murine Virus Antibody Determinations for Rats and Mice in the 4-Week, 2-Year, and Lifetime Inhalation Studies of Ozone and Ozone/NNK

Interval	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
4-Week Studies		
Rats		
Study termination	0/10	None positive
Mice		
Study termination	0/10	None positive
2-Year Studies		
Rats		
Study initiation	0/10	None positive
6 Months	0/16	None positive
12 Months	0/16	None positive
18 Months	0/12	None positive
Study termination	0/10	None positive
Mice		
Study initiation	0/10	None positive
6 Months	0/10	None positive
12 Months	0/10	None positive
18 Months	0/9	None positive
Study termination	0/10	None positive
2-Year Study Ozone/NNK		
Rats		
Study initiation	0/10	None positive
6 Months	0/10	None positive
12 Months	0/10	None positive
18 Months	0/13	None positive
Study termination	3/10[a]	*M. arthritidis*
Lifetime Studies		
Rats		
Study initiation	0/10	None positive
6 Months	0/12	None positive
12 Months	0/12	None positive
18 Months	1/11[b]	H-1 and KRV
Study termination	1/10	*M. arthritidis*
Mice		
Study initiation	0/10	None positive
6 Months	0/10	None positive
12 Months	0/10	None positive
Study termination	2/10	*M. arthritidis*

[a] Two animals positive for *M. arthritidis* were housed in the NNK/air chamber, and one animal was housed in the NNK/Ozone chamber.

[b] Further evaluation by immunofluorescence antibody assay indicated that this was a false positive response.

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS PRINTED AS OF OCTOBER 1994

TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-p-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichlorethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	Telone II® (1,3-Dichloropropene)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl)phosphonium Sulfate & Tetrakis(hydroxymethyl)phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS PRINTED AS OF OCTOBER 1994 (CONT.)

TR No.	CHEMICAL
336	Penicillin VK
337	Nitrofurazone
338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
346	Chloroethane
347	D-Limonene
348	α-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	α-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
384	1,2,3-Trichloropropane
385	Methyl Bromide
386	Tetranitromethane
387	Amphetamine Sulfate
388	Ethylene Thiourea
389	Sodium Azide
390	3,3'-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
392	Chlorinated Water and Chloraminated Water
393	Sodium Fluoride
394	Acetaminophen
395	Probenecid
396	Monochloroacetic Acid
397	C.I. Direct Blue 15
398	Polybrominated Biphenyls
399	Titanocene Dichloride
400	2,3-Dibromo-1-propanol
401	2,4-Diaminophenol Dihydrochloride
402	Furan
403	Resorcinol
404	5,5-Diphenylhydantoin
405	C.I. Acid Red 114
406	γ-Butyrolactone
407	C.I. Pigment Red 3
408	Mercuric Chloride
409	Quercetin
410	Naphthalene
411	C.I. Pigment Red 23
412	4,4-Diamino-2,2-stilbenedisulfonic Acid
413	Ethylene Glycol
414	Pentachloroanisole
415	Polysorbate 80
416	*o*-Nitroanisole
417	*p*-Nitrophenol
418	*p*-Nitroaniline
419	HC Yellow 4
420	Triamterene
421	Talc
422	Coumarin
423	Dihydrocoumarin
424	*o*-Benzyl-*p*-chlorophenol
425	Promethazine Hydrochloride
426	Corn Oil, Safflower Oil, and Tricaprylin
427	Turmeric Oleoresin
428	Manganese (II) Sulfate Monohydrate
430	C.I. Direct Blue 218
431	Benzyl Acetate
432	Barium Chloride Dihydrate
433	Tricresyl Phosphate
434	1,3-Butadiene
437	Hexachlorocyclopentadiene
443	Oxazepam

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge (and while supplies last) from the NTP Central Data Management, NIEHS, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709.

DEPARTMENT OF
HEALTH & HUMAN SERVICES

Public Health Service
National Toxicology Program
Central Data Management
P.O. Box 12233, MD A0-01
Research Triangle Park, NC 27709

SPECIAL FOURTH-CLASS RATE
POSTAGE AND FEES PAID
DHHS/NIH
Permit No. G-763

Official Business
Penalty for Private Use - $300

NIH Publication No. 95-3371
October 1994