

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF TETRAFLUOROETHYLENE
(CAS NO. 116-14-3)
IN F344/N RATS AND B6C3F₁ MICE
(INHALATION STUDIES)

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

April 1997

NTP TR 450

NIH Publication No. 97-3366

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

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. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. 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 E1-02, Research Triangle Park, NC 27709 (919-541-3419). Listings of all published NTP reports and ongoing studies are also available from NTP Central Data Management. The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: <http://ntp-server.niehs.nih.gov>.

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF TETRAFLUOROETHYLENE
(CAS NO. 116-14-3)
IN F344/N RATS AND B6C3F₁ MICE
(INHALATION STUDIES)

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

April 1997

NTP TR 450

NIH Publication No. 97-3366

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

J.H. Roycroft, Ph.D., Study Scientist
 G.A. Boorman, D.V.M., Ph.D.
 D.A. Bridge, B.S.
 J.R. Bucher, Ph.D.
 L.T. Burka, Ph.D.
 R.E. Chapin, Ph.D.
 J.R. Hailey, D.V.M.
 J.K. Haseman, Ph.D.
 G.N. Rao, D.V.M., Ph.D.
 R.C. Sills, D.V.M., Ph.D.
 G.S. Travlos, D.V.M.
 D.B. Walters, Ph.D.
 K.L. Witt, M.S., Oak Ridge Associated Universities

Battelle Pacific Northwest Laboratories

*Conducted 16-day, 13-week, and 2-year studies,
 evaluated pathology findings*

B.J. Chou, D.V.M., Ph.D., Principal Investigator
 J.R. Decker, B.S.
 S.L. Grumbein, D.V.M., Ph.D.
 P.W. Mellick, D.V.M., Ph.D.
 H.A. Ragan, D.V.M.
 S.E. Rowe, D.V.M., M.S.
 R.B. Westerberg, Ph.D.

Experimental Pathology Laboratories, Inc.

Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
 E.T. Gaillard, D.V.M., M.S.

Dynamac Corporation

Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

Analytical Sciences, Inc.

Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
 N.G. Mintz, B.S.
 S. Rosenblum, M.S.

NTP Pathology Working Group

*Evaluated slides, prepared pathology report on rats
 (2 February 1993)*

M.A. Stedham, D.V.M., M.S., Chairperson
 Pathology Associates, Inc.
 J.M. Cullen, V.M.D., Ph.D.
 North Carolina State University
 M.R. Elwell, D.V.M., Ph.D.
 National Toxicology Program
 E.T. Gaillard, D.V.M., M.S.
 Experimental Pathology Laboratories, Inc.
 J.R. Hailey, D.V.M.
 National Toxicology Program
 C.C. Shackelford, D.V.M., M.S., Ph.D.
 National Toxicology Program
 R.C. Sills, D.V.M., Ph.D.
 National Toxicology Program

*Evaluated slides, prepared pathology report on mice
 (3 June 1993)*

M.A. Stedham, D.V.M., M.S., Chairperson
 Pathology Associates, Inc.
 G.A. Boorman, D.V.M., Ph.D.
 National Toxicology Program
 R. Cattley, M.S., V.M.D., Ph.D.
 Chemical Industry Institute of Toxicology
 R.A. Herbert, D.V.M., Ph.D.
 National Toxicology Program
 E.T. Gaillard, D.V.M., M.S.
 Experimental Pathology Laboratories, Inc.
 A. Radovsky, D.V.M., Ph.D.
 National Toxicology Program
 D.D. Sharpnack, D.V.M., M.S.
 National Institute for Occupational Safety and Health
 R.C. Sills, D.V.M., Ph.D.
 National Toxicology Program
 W.M. Witt, D.V.M., Ph.D.
 National Center for Toxicological Research

Biotechnical Services, Inc.

Prepared Technical Report

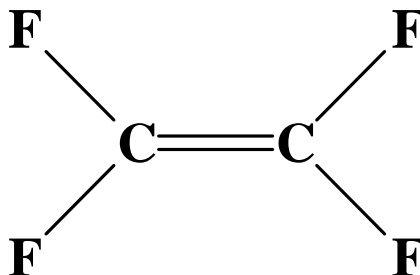
S.R. Gunnels, M.A., Principal Investigator
 L.M. Harper, B.S.
 T.A. King-Hunter, B.S.
 D.C. Serbus, Ph.D.
 S.M. Swift, B.S.

CONTENTS

| | | |
|---|---|------------|
| ABSTRACT | | 5 |
| EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY | | 12 |
| TECHNICAL REPORTS REVIEW SUBCOMMITTEE | | 13 |
| SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS | | 14 |
| INTRODUCTION | | 15 |
| MATERIALS AND METHODS | | 21 |
| RESULTS | | 33 |
| DISCUSSION AND CONCLUSIONS | | 69 |
| REFERENCES | | 75 |
| APPENDIX A | Summary of Lesions in Male Rats in the 2-Year Inhalation Study of Tetrafluoroethylene | 79 |
| APPENDIX B | Summary of Lesions in Female Rats in the 2-Year Inhalation Study of Tetrafluoroethylene | 125 |
| APPENDIX C | Summary of Lesions in Male Mice in the 2-Year Inhalation Study of Tetrafluoroethylene | 165 |
| APPENDIX D | Summary of Lesions in Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene | 209 |
| APPENDIX E | Genetic Toxicology | 253 |
| APPENDIX F | Organ Weights and Organ-Weight-to-Body-Weight Ratios | 255 |
| APPENDIX G | Hematology, Clinical Chemistry, and Urinalysis Results | 263 |
| APPENDIX H | Reproductive Tissue Evaluations and Estrous Cycle Characterization | 273 |
| APPENDIX I | Chemical Characterization and Generation of Chamber Concentrations | 277 |
| APPENDIX J | Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration | 299 |

| | | |
|-------------------|--|------------|
| APPENDIX K | Sentinel Animal Program | 303 |
| APPENDIX L | H-<i>ras</i> Codon 61 Mutation Spectra in Hepatocellular Neoplasms From B6C3F₁ Mice Exposed to Tetrafluoroethylene for 2 Years | 307 |

ABSTRACT



TETRAFLUOROETHYLENE

CAS No. 116-14-3

Chemical Formula: C_2F_4 Molecular Weight: 100.02

Synonyms: Perfluoroethylene; tetrafluoroethene; 1,1,2,2-tetrafluoroethylene; TFE

Tetrafluoroethylene is used in the production of polytetrafluoroethylene (Teflon®) and other polymers. Tetrafluoroethylene was nominated by the National Cancer Institute for toxicity and carcinogenicity studies based on the potential for human exposure to the chemical due to the large production volume and on the lack of adequate data for tetrafluoroethylene in the literature. Male and female F344/N rats and B6C3F₁ mice were exposed to tetrafluoroethylene (98% to 99% pure) by whole body inhalation exposure for 16 days, 13 weeks, or 2 years. Genetic toxicity studies were conducted in mouse peripheral blood erythrocytes.

16-DAY STUDY IN RATS

Groups of five male and five female F344/N rats were exposed to 0, 312, 625, 1,250, 2,500, or 5,000 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week for a total of 12 exposures during a 16-day period. All rats survived to the end of the study. The final mean body weights and body weight gains of males and females exposed to 5,000 ppm were significantly less than those of the controls. The mean body weight gain of females exposed to 2,500 ppm was also significantly less than that of the controls. There were no exposure-related clinical findings in male or female rats. There were no significant differences in hematology parameters that were considered to be related to tetrafluoroethylene expo-

sure. Absolute and relative kidney weights of all exposed groups of males were significantly greater than those of the controls, as were those of females in the 2,500 and 5,000 ppm groups. The absolute kidney weight of females exposed to 1,250 ppm was also significantly greater than that of the controls. The relative liver weights of all exposed groups of males and the absolute liver weights of males in the 625 and 2,500 ppm groups were significantly greater than those of the controls. Increased incidences of renal tubule degeneration occurred in males and females exposed to 625 ppm or greater; this lesion was located predominantly at the corticomedullary junction. The severity of degeneration increased with increasing exposure concentration and was slightly greater in males than females.

16-DAY STUDY IN MICE

Groups of five male and five female B6C3F₁ mice were exposed to 0, 312, 625, 1,250, 2,500, or 5,000 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week for a total of 12 exposures during a 16-day period. All mice survived to the end of the study. Final mean body weights and body weight gains of all exposed groups of mice were similar to those of the controls. There were no exposure-related clinical findings in male or female mice. There were no significant differences in

hematology parameters that were considered to be related to tetrafluoroethylene exposure. The absolute and relative liver weights of females exposed to 5,000 ppm were significantly greater than those of the controls, as was the absolute kidney weight of females in that group and the absolute liver weight of females in the 2,500 ppm group. Renal tubule karyomegaly was observed in male and female mice in the 1,250, 2,500, and 5,000 ppm groups, and the severity of this lesion increased with increasing exposure concentration. Karyomegaly was located predominantly in the inner renal cortex.

13-WEEK STUDY IN RATS

Groups of 10 male and 9 or 10 female F344/N rats were exposed to 0, 312, 625, 1,250, 2,500, or 5,000 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week, for 13 weeks. All rats survived to the end of the study. The final mean body weight and body weight gain of males exposed to 5,000 ppm were significantly less than those of the controls, as was the mean body weight gain of females in this exposure group. There were no clinical findings attributed to exposure to tetrafluoroethylene. Exposure of rats to tetrafluoroethylene resulted in a concentration-dependent normocytic, normochromic, nonresponsive anemia consistent with a secondary hypoproliferative anemia. An exposure concentration-dependent proteinuria also occurred, consistent with renal tubule degeneration observed histopathologically. The absolute and relative liver weights of all exposed groups of males and of females in the 5,000 ppm group were significantly greater than those of the controls. The absolute and relative right kidney weights of males and females exposed to 1,250 ppm or greater and of females in the 625 ppm group were also significantly greater than those of the controls. There were no differences in sperm morphology or vaginal cytology parameters between control and exposed groups of rats. Incidences of renal tubule degeneration in males exposed to 625 ppm or greater and in females exposed to 2,500 or 5,000 ppm were significantly greater than those in the controls. Renal lesions were similar to those observed in the 16-day study and were located predominantly at the corticomedullary junction.

13-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F₁ mice were exposed to 0, 312, 625, 1,250, 2,500, or 5,000 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week, for 13 weeks. All mice survived to the end of the study. Final mean body weights and body weight gains of all exposed groups of male and female mice were generally similar to those of the controls. There were no clinical findings that were considered to be related to tetrafluoroethylene exposure. Exposure of mice to tetrafluoroethylene resulted in a concentration-dependent normocytic, normochromic, nonresponsive anemia, consistent with a secondary hypoproliferative anemia, and in polyuria. Differences in sperm morphology parameters and estrous cycle lengths were not considered to be exposure related. Incidences of karyomegaly of the renal tubule epithelial cells in male and female mice exposed to 1,250 ppm or greater were significantly greater than those in the controls. Karyomegaly was similar to that observed in the 16-day study and was observed primarily in the inner renal cortex.

2-YEAR STUDY IN RATS

Groups of 60 male rats were exposed to 156, 312, or 625 ppm and groups of 60 female rats were exposed to 312, 625, or 1,250 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week, for 104 weeks, with an observation period of 11 days following the final exposure. Ten male and ten female rats from each exposure group were evaluated at 15 months for organ weights and clinical pathology.

Survival, Body Weights, and Clinical Findings

Survival rates of males in the 625 ppm group and of all exposed groups of females were significantly less than those of the controls. Mean body weights of males exposed to 625 ppm were lower than those of the controls from week 81 until the end of the study, and the mean body weight of 1,250 ppm females was slightly lower than that of the controls at the end of the study. The only clinical finding associated with exposure to tetrafluoroethylene was opacity of the eyes in exposed groups of female rats; this change was observed microscopically as cataracts.

Hematology, Clinical Chemistry, and Urinalysis

At the 15-month interim evaluation, there were no differences in hematology, clinical chemistry, or urinalysis parameters that were considered to be related to tetrafluoroethylene exposure.

Pathology Findings

The absolute and relative kidney weights of males exposed to 625 ppm and females exposed to 1,250 ppm and the absolute kidney weight of females exposed to 625 ppm were significantly greater than those of the controls at the 15-month interim evaluation. At 15 months, renal tubule hyperplasia was observed in one male exposed to 312 ppm and one male and one female exposed to 625 ppm; oncocytic hyperplasia was observed in one female exposed to 1,250 ppm. At the end of the study, incidences of renal tubule adenoma were greater in males and females exposed to 312 ppm or greater than those in the controls. This exposure-related increase was confirmed by examination of step sections (extended evaluations). At the end of the study, the incidences of renal tubule hyperplasia in males exposed to 625 ppm and females exposed to 1,250 ppm were significantly greater than those in the controls. The incidences of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) in the extended evaluations and in the standard and extended evaluations (combined) in the 1,250 ppm female group and the 625 ppm male group were significantly greater than those in the controls, and the incidences occurred with significant positive trends. Oncocytic hyperplasia was observed at the end of the study in one male exposed to 312 ppm and in three females exposed to 1,250 ppm. At 15 months and at the end of the study, the incidences of renal tubule degeneration in all exposed groups of males and in females in the 625 and 1,250 ppm groups were greater than those in the controls. Renal tubule degeneration was similar to that observed in the 13-week study and was located predominantly at the corticomedullary junction. The severity of nephropathy generally increased with increasing exposure concentration in male rats at 15 months and 2 years.

The absolute and relative liver weights of females in the 1,250 ppm group and the absolute liver weight of females exposed to 625 ppm were significantly greater than those of the controls at the 15-month interim

evaluation. At 2 years, the incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in males exposed to 312 ppm, the incidences of hepatocellular adenoma and adenoma or carcinoma (combined) in females in all exposed groups, and the incidences of hepatocellular carcinoma in females exposed to 312 or 625 ppm were significantly greater than those in the controls. Also at 2 years, the incidence of hemangiosarcoma in females exposed to 625 ppm was significantly greater than that in the controls. In all exposed groups of males, the incidences of clear cell foci at 15 months were greater than those in the controls; at 2 years, the incidences of eosinophilic foci in all exposed groups of males and the incidences of basophilic and mixed cell foci in males in the 312 and 625 ppm groups were greater than those in the controls. The incidences of mixed cell foci at 15 months in females exposed to 625 or 1,250 ppm and at 2 years in females exposed to 1,250 ppm were also significantly greater than those in the controls. At the end of the 2-year study, increased incidences of cystic degeneration occurred in the liver of all exposed groups of males, and increased incidences of hepatic angiectasis were observed in exposed groups of females.

Incidences of mononuclear cell leukemia in males exposed to 156 ppm and in all exposed groups of females were significantly greater than those in the controls.

Incidences of cataracts in females exposed to 1,250 ppm were greater than those in the controls at the end of the 2-year study.

At the end of the study, there were slight increases in the incidences of testicular interstitial cell adenoma in rats exposed to 312 or 625 ppm.

2-YEAR STUDY IN MICE

Groups of 58 male and 58 female B6C3F₁ mice were exposed to 0, 312, 625, or 1,250 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week, for 95 to 96 weeks. Ten male and ten female mice from each exposure group were evaluated at 15 months for organ weights.

Survival, Body Weights, and Clinical Findings

The survival rates of all exposed groups of males and females were significantly less than those of the controls. Because of the reduced survival due to exposure-related liver neoplasms, the study was terminated during week 96. Mean body weights of exposed groups of males and females were generally similar to those of the controls, except at the end of the study, when they were somewhat less than those of the controls. There were no clinical findings related to tetrafluoroethylene exposure.

Pathology Findings

At the 15-month interim evaluation, there were no differences in absolute or relative kidney, liver, or lung weights between exposed and control groups of mice. At the end of the study, the incidences of multifocal coagulative necrosis of the liver were increased in males in the 625 and 1,250 ppm groups. Also at the end of the study, females in all exposed groups had greater incidences of hematopoietic cell proliferation in the liver than the controls. Angiectasis occurred in all exposed groups of males and females at 15 months and at the end of the study. At the 15-month interim evaluation, hemangiosarcomas were observed in three males exposed to 1,250 ppm and in one female exposed to 312 ppm. The incidences of hemangiosarcoma in all exposed groups of males and females at the end of the study were significantly greater than those in the controls and exceeded the historical chamber control ranges. Also at the end of the study, the incidences of hemangioma in males and females exposed to 312 ppm and in males exposed to 625 ppm were also significantly greater than those in the controls and exceeded the range in historical chamber controls. At 15 months, hepatocellular adenomas and carcinomas occurred in control males and all exposed groups of males and females. Females exposed to 625 or 1,250 ppm had significantly greater incidences of eosinophilic foci than the controls at the 15-month interim evaluation. At the end of the study, the incidences of eosinophilic foci in males exposed to 625 or 1,250 ppm and in females exposed to 312 or 625 ppm were significantly greater than those in the controls. In male and female mice, increased incidences of a variety of hepatocellular neoplasms, including adenomas, multiple adenomas, carcinomas, and multiple carcinomas, were considered related to tetrafluoroethylene exposure.

At the end of the study, the incidences of histiocytic sarcoma (all organs) in all exposed groups of males and females were significantly greater than those in the controls and exceeded the historical control ranges for all organs. The greatest incidences of histiocytic sarcomas were observed in the liver and lung, but these neoplasms were also observed in the spleen, lymph nodes, bone marrow, and kidney.

Significantly increased incidences of renal tubule dilatation (males) and karyomegaly (males and females), located predominantly in the inner cortex, were observed in mice exposed to 625 or 1,250 ppm at 15 months. At the end of the study, the increased incidences of dilatation and karyomegaly in all exposed groups of males and of karyomegaly in 1,250 ppm females were generally significant.

Incidences of hematopoietic cell proliferation in the spleen of all exposed groups of males and females were significantly greater than those in the controls at the end of the study. Additionally, the severity of this lesion increased with increasing exposure concentration.

GENETIC TOXICOLOGY

No increases in the frequency of micronucleated erythrocytes were observed in peripheral blood samples obtained from male and female mice at the end of the 13-week inhalation study of tetrafluoroethylene.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of tetrafluoroethylene in male F344/N rats based on increased incidences of renal tubule neoplasms (mainly adenomas) and hepatocellular neoplasms. There was *clear evidence of carcinogenic activity* of tetrafluoroethylene in female F344/N rats based on increased incidences of renal tubule neoplasms, liver hemangiosarcomas, hepatocellular neoplasms, and mononuclear cell leukemia. There was *clear evidence of carcinogenic activity* of tetrafluoroethylene in male and female B6C3F₁ mice based on increased incidences of liver hemangiomas and hemangiosarcomas, hepatocellular neoplasms, and histiocytic sarcomas.

Slight increases in the incidences of mononuclear cell leukemia and testicular interstitial cell adenomas in male rats may have been related to exposure to tetrafluoroethylene.

Exposure of rats to tetrafluoroethylene resulted in increased incidences of renal tubule hyperplasia and degeneration in males and females, increased severity

of kidney nephropathy in males, and increased incidences of liver angiectasis and cataracts in females. Exposure of mice to tetrafluoroethylene resulted in increased incidences of hematopoietic cell proliferation of the liver in females, liver angiectasis in males and females, renal tubule dilatation in males, renal tubule karyomegaly in males and females, and splenic hematopoietic cell proliferation in males and females.

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

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Tetrafluoroethylene

| | Male F344/N Rats | Female F344/N Rats | Male B6C3F₁ Mice | Female B6C3F₁ Mice |
|--------------------------------|--|--|--|--|
| Exposure Concentrations | 0, 156, 312, or 625 ppm | 0, 312, 625, or 1,250 ppm | 0, 312, 625, or 1,250 ppm | 0, 312, 625, or 1,250 ppm |
| Body Weights | 625 ppm group lower than controls | 1,250 ppm group slightly lower than controls at end of study | Exposed groups lower than controls at end of study | Exposed groups lower than controls at end of study |
| Survival Rates | 17/50, 12/50, 17/50, 1/50 (2 years) | 28/50, 16/50, 15/50, 18/50 (2 years) | 38/48, 11/48, 2/48, 1/48 (22 months) | 36/48, 4/48, 6/48, 4/48 (22 months) |
| Nonneoplastic Effects | <u>Kidney (renal tubule)</u> : hyperplasia (single sections - 1/50, 1/50, 1/50, 6/50; single and step sections - 7/50, 11/50, 7/50, 24/50); degeneration (2/50, 20/50, 50/50, 49/50); severity of nephropathy (2.3, 1.9, 2.7, 3.5) | <u>Kidney (renal tubule)</u> : hyperplasia (single sections - 1/50, 3/50, 6/50, 12/50; single and step sections - 3/50, 6/50, 11/50, 25/50); degeneration (0/50, 0/50, 35/50, 46/50) <u>Liver</u> : angiectasis (0/50, 9/50, 9/50, 14/50) <u>Eye</u> : cataracts (15/50, 4/50, 10/50, 45/50) | <u>Liver</u> : angiectasis (0/48, 6/48, 10/48, 13/48) <u>Kidney (renal tubule)</u> : dilatation (0/48, 4/48, 16/48, 36/48); karyomegaly (1/48, 2/48, 10/48, 28/48) <u>Spleen</u> : hematopoietic cell proliferation (14/48, 32/48, 41/46, 42/46) | <u>Liver</u> : hematopoietic cell proliferation (3/48, 19/48, 13/47, 15/47); angiectasis (1/48, 9/48, 6/47, 4/47) <u>Kidney (renal tubule)</u> : karyomegaly (0/48, 0/48, 0/47, 38/48) <u>Spleen</u> : hematopoietic cell proliferation (18/48, 39/48, 41/46, 41/47) |

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

| | Male F344/N Rats | Female F344/N Rats | Male B6C3F ₁ Mice | Female B6C3F ₁ Mice |
|---|--|---|---|---|
| Neoplastic Effects | <p><u>Kidney (renal tubule):</u> adenoma (single sections - 0/50, 0/50, 6/50, 3/50; single and step sections - 2/50, 4/50, 9/50, 13/50); carcinoma (single sections - 1/50, 0/50, 2/50, 0/50; single and step sections - 1/50, 1/50, 2/50, 0/50); adenoma or carcinoma (single sections - 1/50, 0/50, 6/50, 3/50; single and step sections - 3/50, 5/50, 9/50, 13/50)</p> <p><u>Liver:</u> hepatocellular carcinoma (1/50, 1/50, 10/50, 3/50); hepatocellular adenoma or carcinoma (4/50, 7/50, 15/50, 8/50)</p> | <p><u>Kidney (renal tubule):</u> adenoma (single sections - 0/50, 3/50, 1/50, 3/50; single and step sections - 0/50, 3/50, 3/50, 8/50); carcinoma (single sections - 0/50, 0/50, 0/50, 2/50; single and step sections - 0/50, 0/50, 0/50, 3/50); adenoma or carcinoma (single sections - 0/50, 3/50, 1/50, 5/50; single and step sections - 0/50, 3/50, 3/50, 10/50)</p> <p><u>Liver:</u> hemangiosarcoma (0/50, 0/50, 5/50, 1/50); hepatocellular adenoma (0/50, 4/50, 5/50, 6/50); hepatocellular carcinoma (0/50, 4/50, 9/50, 2/50); hepatocellular adenoma or carcinoma (0/50, 7/50, 12/50, 8/50)</p> <p><u>Mononuclear cell leukemia:</u> (16/50, 31/50, 23/50, 36/50)</p> | <p><u>Liver:</u> hemangioma (0/48, 10/48, 5/48, 2/48); hemangiosarcoma (0/48, 21/48, 27/48, 37/48); hemangioma or hemangiosarcoma (0/48, 26/48, 30/48, 38/48); hepatocellular carcinoma (11/48, 20/48, 33/48, 26/48); hepatocellular adenoma or carcinoma (26/48, 34/48, 39/48, 35/48)</p> <p><u>Hematopoietic system (all organs):</u> histiocytic sarcoma (0/48, 12/48, 7/48, 7/48)</p> | <p><u>Liver:</u> hemangioma (0/48, 5/48, 2/47, 1/47); hemangiosarcoma (0/48, 27/48, 27/47, 34/47); hemangioma or hemangiosarcoma (0/48, 31/48, 28/47, 35/47); hepatocellular carcinoma (4/48, 28/48, 22/47, 20/47); hepatocellular adenoma or carcinoma (17/48, 33/48, 29/47, 28/47)</p> <p><u>Hematopoietic system (all organs):</u> histiocytic sarcoma (1/48, 21/48, 19/47, 18/48)</p> |
| Uncertain Findings | <p><u>Mononuclear cell leukemia:</u> (34/50, 43/50, 38/50, 31/50)</p> <p><u>Testis:</u> interstitial cell adenoma (39/50, 40/50, 48/50, 47/50)</p> | None | None | None |
| Level of Evidence of Carcinogenic Activity | Clear evidence | Clear evidence | Clear evidence | Clear evidence |
| Genetic Toxicology | | | | |
| | Micronucleated erythrocytes | | | |
| | Mouse peripheral blood <i>in vivo</i> : | Negative | | |

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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 tetrafluoroethylene on December 5, 1995, 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.

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

Irma Russo, M.D.
Fox Chase Cancer Center
Philadelphia, PA

Gary P. Carlson, Ph.D., Principal Reviewer
School of Health Sciences
Purdue University
West Lafayette, IN

Louise Ryan, Ph.D., Principal Reviewer
Division of Biostatistics
Dana-Farber Cancer Institute
Boston, MA

Thomas L. Goldsworthy, Ph.D.
Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

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

Robert LeBoeuf, Ph.D.
Corporate Professional and Regulatory Services
Human Safety Department
The Procter & Gamble Company
Cincinnati, OH

Frederick L. Tyson, Ph.D.
St. Mary's Hospital and Medical Center
Cancer Research Institute
Grand Junction, CO

Janardan K. Reddy, M.D., Principal Reviewer*
Department of Pathology
Northwestern University Medical School
Chicago, IL

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

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 5 December 1995, the draft Technical Report on the toxicology and carcinogenesis studies of tetrafluoroethylene received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.H. Roycroft, NIEHS, introduced the toxicology and carcinogenesis studies of tetrafluoroethylene by describing the uses of the chemical, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplasms and nonneoplastic lesions in male and female rats and mice. Dr. R.C. Sills, NIEHS, presented data from ongoing molecular biology studies characterizing the *H-ras* codon 61 mutation spectra in hepatocellular neoplasms from control B6C3F₁ mice and mice exposed to tetrafluoroethylene for 2 years. Data were also presented comparing the mutation profiles of hepatocellular neoplasms from the current study with those from the NTP study of tetrachloroethylene. The proposed conclusions for the 2-year studies were *clear evidence of carcinogenic activity* in male and female F344/N rats and male and female B6C3F₁ mice.

Dr. Ryan, a principal reviewer, agreed with the proposed conclusions. She inquired as to whether short-burst, high exposures had been considered in the study design as being more similar to a typical occupational exposure. Dr. Roycroft replied that this regimen had not been considered; the 6-hour-per-day, 5-day-per-week design used was similar to continuous exposure in a workplace situation. Dr. Ryan asked whether there was information on the degree of human exposure encountered through spills or leaks. Dr. Roycroft noted that tetrafluoroethylene is produced and maintained in closed-capture systems, no occupational exposure limits have been established, and no information on spills or leaks has been found.

Dr. Carlson, the second principal reviewer, agreed with the proposed conclusions. He observed that the

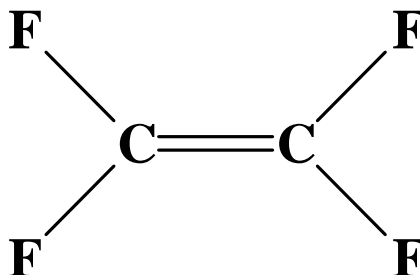
numbers of clinical pathology measurements appeared to be decreased from past reports and, in view of the types of lesions seen, wondered why. Dr. Roycroft responded that, in actuality, there were more measurements; in addition to standard time points and collections at 13 weeks, clinical pathology analyses were performed on animals killed at 15 months. Dr. Carlson said a sentence needed to be added to the Abstract regarding the reason for terminating the mouse study early. Dr. Roycroft agreed.

Dr. Reddy, the third principal reviewer, was unable to attend the meeting but had submitted his review, which Dr. L.G. Hart, NIEHS, read into the record. Dr. Reddy agreed with the proposed conclusions and found the report acceptable.

There was some discussion about the mixing of rats and mice in exposure chambers. Dr. Goldsworthy asked if hormonal alterations were seen. Dr. Roycroft said that hormone measurements were not made, but if a chemical to be studied was known to affect hormone levels, then separate housing might have to be considered. Dr. G.N. Rao, NIEHS, noted that rats and mice are produced in the same rooms so each species is accustomed to the other's presence; he pointed out that each inhalation chamber is by itself a room, and within the chamber, rats and mice are not side by side in cages, but rather in racks. This provides more efficient and economical use of available space. Dr. LeBoeuf commended the NTP for the molecular biology studies evaluating the *H-ras* activation profiles and noted the usefulness of such studies for risk assessment purposes.

Dr. Ryan moved that the Technical Report on tetrafluoroethylene be accepted with the revisions discussed and with the conclusions as written for male and female rats and male and female mice, *clear evidence of carcinogenic activity*. Dr. Carlson seconded the motion, which was accepted unanimously with seven votes.

INTRODUCTION



TETRAFLUOROETHYLENE

CAS No. 116-14-3

Chemical Formula: C₂F₄ Molecular Weight: 100.02

Synonyms: Perfluoroethylene; tetrafluoroethene; 1,1,2,2-tetrafluoroethylene; TFE

CHEMICAL AND PHYSICAL PROPERTIES

Tetrafluoroethylene is a colorless, odorless gas with a melting point of -142.5°C , a boiling point of -76.3°C , a specific gravity of 1.52 at -76°C , a vapor density of 3.87, and a vapor pressure of 428.4 mm Hg. It is insoluble in water. Tetrafluoroethylene is a highly flammable gas that reacts with oxidants and has a flash point of less than 0°C and an autoignition temperature of 188°C . The lower explosion limit of the chemical is 10% v/v and the upper explosion limit is 50% v/v. Tetrafluoroethylene can dimerize under appropriate temperature and pressure conditions to a stable perfluorocyclobutene and polymerizes easily in the absence of inhibitors or in the presence of heat or oxygen (IARC, 1979; Bretherick, 1985; Weast, 1985; NFPA, 1986; *Material Safety Data Sheet Collection*, 1993; HSDB, 1995).

PRODUCTION, USE, AND HUMAN EXPOSURE

Tetrafluoroethylene is generally produced by the pyrolysis of chlorodifluoromethane or trifluoro-

methane (IARC, 1979; Hawley, 1981; *Material Safety Data Sheet Collection*, 1993). Although tetrafluoroethylene was reported to the United States International Trade Commission for the year 1992, there was no estimate of the annual production (USITC, 1994). The most recent estimated United States annual production figure of 28 million pounds was reported in 1978. Virtually all of the tetrafluoroethylene produced in the United States is used in the synthesis of Teflon®, one of several polytetrafluoroethylene polymers (Kennedy, 1990). Because of its chemical properties, tetrafluoroethylene is produced and maintained in closed-capture systems. For this reason, human exposure would be primarily through leakage from these systems or through contact with one of several decomposition products of high-temperature processing or pyrolysis of Teflon® and other polymers (Kennedy, 1990). According to the National Occupational Exposure Survey, 14,963 male and 325 female workers were potentially exposed to tetrafluoroethylene from the years 1981 to 1983 (NIOSH, 1990). Currently, no occupational exposure limits have been established. There were no reports in the literature of the detection of tetrafluoroethylene in ambient air, drinking water, or wastewater, and the chemical is not known to occur naturally.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

Dilley *et al.* (1974) reported the urinary excretion of fluoride ion following a 30-minute exposure of Sprague-Dawley rats to 3,500 ppm tetrafluoroethylene by inhalation. A significant increase in fluoride excretion occurred on days 6, 13, and 14 following exposure. Urinary creatinine, potassium, and volume were significantly increased following exposure to tetrafluoroethylene. Increased urinary fluoride was observed in male rats and hamsters exposed to 100 to 2,500 ppm tetrafluoroethylene by inhalation for 2 weeks; however, urinary fluoride concentrations returned to normal upon cessation of exposure (Kennedy, 1990). Male and female rats and hamsters exposed to 200 to 2,000 ppm tetrafluoroethylene by inhalation for 18 weeks displayed an exposure-related increase in urinary fluoride (Kennedy, 1990). Ding *et al.* (1980) exposed rabbits to 1,000 ppm tetrafluoroethylene for 60 minutes via a face mask and determined alveolar absorption to be 6.76%. Animals were evaluated during exposure and for a period of up to 75 minutes after exposure ended. The lung, bone, and kidney had the highest fluoride content of the sites examined.

As a part of an investigation of tetrafluoroethylene-induced nephrotoxicity, Odum and Green (1984) demonstrated that liver slices from Wistar rats metabolized tetrafluoroethylene to *S*-(1,1,2,2-tetrafluoroethyl)glutathione. The reaction was catalyzed by both microsomal and cytosolic glutathione *S*-transferases, with the rate of formation with microsomal fractions occurring at four times the rate with cytosol fractions. These same authors also identified the cysteinylglycine and cysteine conjugates of tetrafluoroethylene in bile from rats exposed to 6,000 ppm tetrafluoroethylene for 6 hours. Oral administration of the cysteine conjugate [*S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine] to rats caused renal damage identical to that caused by tetrafluoroethylene. Cytochrome P₄₅₀ oxidation did not appear to be involved in tetrafluoroethylene metabolism.

Cysteine conjugates of the nephrotoxins hexachlorobutadiene, tetrafluoroethylene, and hexa-

fluoropropane, together with those of trichloroethylene and perchloroethylene, have been chemically synthesized, and a relationship between their structures, nephrotoxicity, and mutagenicity *in vitro* has been determined (Green and Odum, 1985). All of the conjugates had a marked effect on the uptake of both the organic anion *p*-aminohippuric acid and the cation tetraethylammonium bromide into rat kidney slices, suggesting activation of the conjugates in the slices to a toxic species which interferes with ion transport. This observation is consistent with the known nephrotoxicity of hexachlorobutadiene, tetrafluoroethylene, and hexafluoropropene *in vivo*. Each of the conjugates was found to be metabolized by rat kidney slices and by semi-purified rat kidney β -lyase to pyruvate, ammonia, and an unidentified reactive metabolite (postulated at that time to be a thiol). When semi-purified β -lyase was used, stoichiometric amounts of pyruvate and ammonia were produced. Although all of the conjugates were activated by β -lyase and had a similar effect on ion transport, their mutagenicity differed markedly. The conjugates of hexachlorobutadiene, trichloroethylene, and tetrachloroethylene were mutagenic in the Ames bacterial mutation assay when activated by rat kidney S9. Metabolic cofactors were not required, suggesting that activation was due to the enzyme β -lyase. In the same assay, conjugates of tetrafluoroethylene and hexafluoropropene were not mutagenic either in the presence or absence of rat kidney S9 and cofactors. With a limited number of cysteine conjugates, a clear distinction was identified between the conjugates of fluoroalkenes that were similarly nephrotoxic but not mutagenic. The mutagenicity of the cysteine conjugate of hexachlorobutadiene is consistent with the known renal carcinogenicity of this chemical.

Since the initial observations by Odum and Green (1984), a number of laboratories have investigated the *in vitro* and *in vivo* metabolism of nephrotoxic haloalkenes (including tetrafluoroethylene) and their cysteine conjugates in order to determine the mechanism of haloalkene-induced nephrotoxicity. The bioactivation of the haloalkenes likely begins with glutathione conjugation in the liver. These conjugates are primarily excreted via the bile in the small intestine, where biliary and intestinal peptidases degrade them to cysteine-*S*-conjugates which may be

reabsorbed from the small intestine. Glutathione conjugates released into the general circulation from the liver are hydrolyzed to the cysteine- *S*-conjugates by renal peptidases and are bioactivated by renal β -lyase to unstable, reactive thiols. Commandeur *et al.* (1988) administered tetrafluoroethylene-mercapturic acid intraperitoneally to Wistar rats at doses of 50 $\mu\text{mol/kg}$ body weight or greater. After 48 hours, nephrotoxicity, but not liver toxicity, ensued, as evidenced by increased blood urea nitrogen, urinary protein and glucose, and relative kidney weights. Excretion of difluoroacetic acid was dose related, represented 10% of the dose of 50 $\mu\text{mol/kg}$ body weight, and occurred primarily during the first 24 hours. Difluoroacetic acid, pyruvate, hydrogen sulfide, and thiosulfate were formed during *in vitro* metabolism of the tetrafluoroethylene-cysteine conjugate by rat renal cytosol. Difluoroacetic acid formation was inhibited by aminooxyacetic acid, a β -lyase inhibitor, which points to a β -lyase dependency for its formation. This suggests that the tetrafluoroethylene-cysteine conjugate is bioactivated to a significant extent to difluorothionacyl fluoride and is subsequently metabolized to difluoroacetic acid (Commandeur *et al.*, 1988). Male Wistar rats were also injected with 50 μmol tetrafluoroethylene-cysteine conjugate/kg body weight. After 48 hours, 4% of the dose was excreted as the corresponding mercapturic acid. In order to evaluate the enzymatic activation or deactivation of various halogenated cysteine conjugates and mercapturic acids, rat liver and kidney cytosol (activation) and microsomes (deactivation) were incubated with the tetrafluoroethylene-cysteine conjugates and mercapturic acid or various chloro- and bromofluoro cysteine conjugates and mercapturic acids. Tetrafluoroethylene-cysteine conjugate incubated with β -lyase or tetrafluoroethylene-mercapturic acid with *N*-deacetylase demonstrated a greater activation activity with kidney cytosol than with liver. In addition, tetrafluoroethylene-cysteine conjugates and mercapturic acids demonstrated greater activity than chloro- or bromofluoro compounds. Deactivation as evidenced by *N*-acetyltransferase activity (microsomes) was greater with the chloro- and bromofluoro compounds than with tetrafluoroethylene conjugates or mercapturic acid. Deactivation activity was greatest with renal microsomes for both tetrafluoroethylene compounds (Commandeur *et al.*, 1991). The same laboratory also demonstrated that when

isolated proximal tubule cells from Wistar rat kidneys were incubated with 100 μmol of tetrafluoroethylene-cysteine conjugates or mercapturic acid and other chloro- and bromofluoro compounds, tetrafluoroethylene compounds were more toxic, as evidenced by their ability to inhibit α -methylglucose uptake by the cells (Boogaard *et al.*, 1989). The cytotoxicity induced by both tetrafluoroethylene compounds was inhibited by aminooxyacetic acid; however, only the toxicity induced by tetrafluoroethylene-mercapturic acid could be inhibited by probenecid, suggesting that the two tetrafluoroethylene compounds are transported by a different carrier system. Tetrafluoroethylene cysteine accumulated in the proximal tubule cells on exposure to either tetrafluoroethylene-cysteine conjugate or tetrafluoroethylene-mercapturic acid (Boogaard *et al.*, 1989). Commandeur *et al.* (1989) demonstrated that the tetrafluoroethylene-cysteine conjugate may be bioactivated in the rat kidney and, to a lesser extent, in the rat liver to electrophilic thioacylating reactive intermediates, probably thionacetyl fluorides, which are the putative reactive intermediates for tetrafluoroethylene-induced nephrotoxicity.

A number of studies have demonstrated that the tetrafluoroethylene-cysteine conjugate and tetrafluoroethylene-derived thionacetyl fluorides affect rat and rabbit kidney macromolecules. Using isolated rat kidney mitochondria, Hayden and Stevens (1990) demonstrated that tetrafluoroethylene-cysteine conjugate was a good substrate for the mitochondrial β -lyase, inhibited ADP-stimulated respiration (primarily stage III), was covalently bound to macromolecules (blocked by aminooxyacetic acid), and was metabolized by mitochondria. Rabbit renal proximal tubules incubated with 25 μmol tetrafluoroethylene-cysteine conjugate produced similar results, including a sevenfold increase in lipid peroxidation (Groves *et al.*, 1993). Although the majority of binding in rat mitochondria is associated with the protein fraction, Hayden *et al.* (1992) have shown that significant binding occurs in the lipid fraction, and the major phospholipid adduct is the thioamide adduct of phosphatidylethanolamine.

Harris *et al.* (1992) administered 110 mmol tetrafluoroethylene-cysteine conjugate intraperito-

neally to male Fischer rats. After 1 hour, kidneys were removed, and cytosolic, microsomal, and mitochondrial fractions were obtained. The single, stable amino acid adduct formed with renal protein was *N*-(difluorothioacetyl)lysine. Urine taken from bladders at necropsy was analyzed for metabolites. Mercapturic acids, dihaloacetic acids, and inorganic fluoride were present, as in previously reported studies. In a similar experiment, Hayden *et al.* (1991a) intraperitoneally administered 30 mg tetrafluoroethylene-cysteine conjugate/kg body weight to male Sprague-Dawley rats. The resulting kidney toxicity showed a good correlation with metabolite binding in that difluorothioamide adducts and toxicity were localized to the medullary ray, which contains the S3C and S3M segments of the proximal tubule. Cell death occurred only in cells containing adducts.

Protein adducts such as *N*-acetyl-*N*-(difluorothioacetyl)lysine (Hayden *et al.*, 1991b) from rat renal subcellular fractions and *N*-(difluorothioacetyl)-*S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine (Commandeur *et al.*, 1989) from rat renal and liver subcellular fractions have been isolated. Bruschi *et al.* (1993) have demonstrated that as many as five mitochondrial proteins are modified following intraperitoneal injection of male F344/N rats with 30 mg tetrafluoroethylene-cysteine conjugate/kg body weight; modification also occurs at the time of formation of the *N*-acetyl-*N*-(difluorothioacetyl)lysine adduct. Two of the proteins have been identified as the heat shock proteins HSP60 (P1 protein) and HSP70-like protein (mortalin). HSP70 and HSP70 mRNA are increased in porcine renal epithelial cell cultures when tetrafluoroethylene-cysteine conjugate is added (Chen *et al.*, 1992). Lock and Schnellmann (1990), using *in vitro* studies with rat renal mitochondrial or cytosolic preparations, have demonstrated that the addition of tetrafluoroethylene-cysteine conjugate inhibits lipoyl dehydrogenase and glutathione reductase activity, respectively.

Humans

No studies on the absorption, distribution, metabolism, or excretion of tetrafluoroethylene by humans were found in the available literature (National Library of Medicine, 1995).

TOXICITY

Experimental Animals

Sakharova and Tolgskaya (1977) reported LC₅₀ values of 31,000 to 32,000 ppm for rats; 35,000 ppm for mice; and 28,000 ppm for guinea pigs following 4-hour inhalation exposures to tetrafluoroethylene. Kennedy (1990) reported a similar 4-hour LC₅₀ of 28,500 ppm for hamsters. Zhemerdei (1958) reported that the lowest concentration of tetrafluoroethylene that caused mortality in rabbits was 40,000 ppm.

Odum and Green (1984) exposed male Wistar rats to tetrafluoroethylene for 6 hours at concentrations of 1,000, 2,000, 3,000, 4,000, and 6,000 ppm to evaluate the potential renal toxicity of the compound. Blood urea nitrogen values, urine volume and urinary excretion of glucose, and alkaline phosphatase and γ -glutamyltranspeptidase activities were significantly increased in rats exposed to 4,000 or 6,000 ppm. These changes are indicative of renal tubule damage. Histopathologic evaluations of the kidneys were conducted on rats in the control and 6,000 ppm groups. All exposed rats exhibited marked renal necrosis involving the pars recta of the proximal tubules. In addition, there were intertubule calcified deposits in the medulla. The no-observable-effect level for kidney toxicity was 2,000 ppm. As in previously discussed studies, oral exposure to tetrafluoroethylene-cysteine conjugate caused renal damage identical to that observed following exposure to tetrafluoroethylene via inhalation.

A number of industry-sponsored studies with tetrafluoroethylene have been conducted but not published. Kennedy (1990) has provided a brief review of some of these studies. Because few repeated-exposure studies with tetrafluoroethylene are presented in the available published literature, several of the studies are summarized here. Syrian hamsters and male Crl:CD rats were exposed to tetrafluoroethylene for 6 hours per day, 5 days per week, for 2 weeks at exposure concentrations of 0, 100, 500, 1,000, and 2,500 ppm. Some of the animals were evaluated at the end of the exposure period, and the remaining animals were evaluated following a 14-day recovery period. No clinical signs of toxicity were observed in either species. There were no significant differences in hamster

absolute or relative organ weights (liver or kidney) at either evaluation period. After the tenth exposure, absolute and relative kidney and liver weights were significantly increased in rats exposed to 2,500 ppm tetrafluoroethylene; however, following the recovery period, kidney and liver weights were similar to those of the control group. Exposure-related kidney lesions in rats exposed to 2,500 ppm were characterized by swelling of the tubular epithelial cells and dilation of the tubular lumen. Sparse cellular degeneration was observed and localized to the juxtamedullary cortex. No significant lesions were apparent following the 14-day recovery period. No significant histopathologic changes were observed in tetrafluoroethylene-exposed hamsters after the tenth exposure; however, following the 14-day recovery period, testicular atrophy was observed in the 2,500 ppm group. This effect consisted of degeneration and sloughing of germinal epithelial cells, resulting in an absence or reduction in the number of mature sperm in both the testes and epididymides, and the presence of degenerative germ cells sloughed into the lumen of the epididymal ducts. These effects were noted less frequently in animals exposed to lower concentrations.

Kennedy (1990) summarized the results of a study in which male and female rats and hamsters were exposed to 200, 600, or 2,000 ppm tetrafluoroethylene for 6 hours per day, 5 days per week, for 18 weeks. Hamsters appeared to be less affected than rats in that there was no reduction in the rate of weight gain in any exposed group of hamsters, while rats exposed to 2,000 ppm exhibited a reduction in the rate of weight gain. Additionally, toxic nephrosis associated with the straight portions of the proximal convoluted tubules was observed in rats exposed to 600 or 2,000 ppm tetrafluoroethylene. This was accompanied by increased urine volume and urine fluoride concentrations and decreased urine creatinine concentrations. The effects were more pronounced in female rats than in male rats. No effects on the kidneys of exposed groups of hamsters were observed; however, testicular atrophy similar to that reported in the 14-day study in male Syrian hamsters and Crl:CD rats was observed.

Although a number of animal studies have been performed with pyrolysis products of tetrafluoroethylene polymers, they are not reported here

because these products are mixtures of a number of chemical vapors and particulates, and any observable effects cannot be solely attributed to the inhalation of tetra-fluoroethylene monomer.

Humans

Epidemiological studies relative to exposure to tetrafluoroethylene alone were not found in the literature (National Library of Medicine, 1995). Although monomeric tetrafluoroethylene is generally considered an irritant to the eyes and respiratory tract, there is a paucity of firm toxicologic data. There are considerable data in the literature on human exposure to pyrolysis products of tetrafluoroethylene polymers. However, those studies are not considered here because toxic effects from exposure to the polymer fumes cannot be attributed solely to inhalation of tetrafluoroethylene monomer. Following exposure to pyrolysis products of tetrafluoroethylene, influenza-like symptoms commonly referred to as “polymer-fume fever” do occur. The symptoms include fever, chills, headaches, dizziness, nausea, weakness, cough, and rigor-like shaking of the limbs (IARC, 1979; Gosselin *et al.*, 1984; *Material Safety Data Sheet Collection*, 1993; *Patty's Industrial Hygiene and Toxicology*, 1994). The symptoms are transient if the oxygen supply to the subject is increased and the subject is removed from the contaminated environment.

S-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine and other cysteine and glutathione conjugates such as *S*-(1,2-dichlorovinyl)glutathione, *S*-(1,2-dichlorovinyl)-*L*-cysteine, *S*-(1,2,3,4,4-pentachlorobutadienyl)-*L*-cysteine, and *S*-(2-chloro-1,1,2-trifluoroethyl)-*L*-cysteine were found to be toxic to human proximal tubule cells in culture, as indicated by the release of lactate dehydrogenase (Chen *et al.*, 1990). Aminoxyacetic acid protected the cells from the toxicity of all the conjugates and inhibited the covalent binding of the sulfur-containing metabolites to cellular macromolecules and thus prevented cell death.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No information on the reproductive or developmental toxicity of tetrafluoroethylene in

experimental animals or in humans was found in a search of the available literature (National Library of Medicine, 1995).

CARCINOGENICITY

No information on the carcinogenicity of tetrafluoroethylene in experimental animals or in humans was found in a search of the available literature (National Library of Medicine, 1995). Tetrachloroethylene, which is structurally similar to tetrafluoroethylene, produced hepatocellular carcinomas in male and female B6C3F₁ mice (NCI, 1977). Male mice were administered 450 or 900 mg tetrachloroethylene/kg body weight in corn oil by gavage for 11 weeks, then 550 or 1,100 mg/kg for 67 weeks. Female mice were administered 300 or 600 mg/kg tetrachloroethylene in corn oil by gavage for 11 weeks, then 400 or 800 mg/kg for 67 weeks. Exposure of male and female F344/N rats to 200 or 400 ppm tetrachloroethylene by inhalation for 2 years resulted in increased incidences of renal tubule adenoma or adenocarcinoma in males and increased incidences of mononuclear cell leukemia in males all these tests were conducted with and without Aroclor-induced liver S9 enzymes.

STUDY RATIONALE

Tetrafluoroethylene was nominated to the NTP by the National Cancer Institute for testing following a review of related monomers and polymers. The

and females. (NTP, 1986). In the same inhalation study, mice exposed to 100 or 200 ppm for 2 years developed increased incidences of hepatocellular adenoma (males) and carcinoma (males and females).

GENETIC TOXICOLOGY

Kennedy (1990) reported that tetrafluoroethylene was not mutagenic in *Salmonella typhimurium* with or without metabolic activation.

Cysteine conjugates of tetrafluoroethylene were not mutagenic in the *Salmonella* assay, with or without rat kidney S9 (Green and Odum, 1985).

The structurally similar tetrachloroethylene was not mutagenic in *Salmonella* (Haworth *et al.*, 1983), gave equivocal results in the mouse lymphoma mutagenicity assay with L5178Y mouse lymphoma cells (Myhr *et al.*, 1990), and did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells (Galloway *et al.*, 1987);

nomination was based on the potential for human exposure to the chemical due to the large production volume and on the lack of adequate test data for tetrafluoroethylene. Inhalation was chosen as the route of exposure because human exposure occurs primarily via this route. Sixteen-day, 13-week, and 2-year whole body inhalation studies were performed in male and female F344/N rats and B6C3F₁ mice.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF TETRAFLUOROETHYLENE

Tetrafluoroethylene was obtained from SCM Specialty Chemicals (Gainesville, FL) in one lot (10271), which was used for the identity and purity analyses conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix I). Tetrafluoroethylene was also obtained from ICI Americas, Inc. (Bayonne, NJ), in five lots. The study laboratory assigned a lot number to each shipment of as many as 12 cylinders. Lot 12017-8 was used during the 16-day studies and during most of the 13-week studies; lot 12017-77 was used during the remainder of the 13-week studies; and lots 12438-6, 12438-79, and 12438-92 were used during the 2-year studies. Identity and purity analyses were also conducted by the study laboratory on each lot used during the studies. Reports on analyses performed in support of the tetrafluoroethylene studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Each lot of the chemical, a colorless gas, was identified as tetrafluoroethylene by the analytical chemistry of the study laboratory using infrared spectroscopy and the purity of each lot was determined by gas chromatography. Gas chromatography of lot 10271 indicated one major peak and one impurity with an area of 0.1% relative to the major peak. In addition, analysis for *d*-limonene, which is added to tetrafluoroethylene as a stabilizer, was conducted by the analytical chemistry laboratory using gas chromatography and indicated 0.03% ± 0.00% *d*-limonene. Gas chromatography conducted by the study laboratory for each cylinder of each lot used during the studies indicated that perfluorocyclobutane was present at concentrations less than or equal to 462 ppm (0.05%) for the 16-day studies and 2,604 ppm (0.26%) for the 13-week studies. During the 2-year studies, gas chromatography indicated peaks for perfluorocyclobutane with areas less than or equal to 1.21% relative to the major peak. Gas chromatography conducted by the study laboratory for each cylinder of each lot used during the 13-week studies indicated that *d*-limonene was present

at concentrations less than or equal to 2,837 ppm (0.28%). During the 2-year studies, gas chromatography of lots 12438-6, 12438-79, and 12438-92 indicated peaks for *d*-limonene with areas less than or equal to 0.56% relative to the major peak. The manufacturer indicated that the following impurities were also present at concentrations less than or equal to 1.7 ppm: trifluoroethylene, methylene fluoride, vinyl fluoride, and vinylidene fluoride. The overall purity of lots 12017-8, 12017-77, and 12438-92 was determined to be greater than 99%. The overall purity of lots 12438-6 and 12438-79 was determined to be greater than 98%.

The manufacturer indicated that tetrafluoroethylene would be stable for up to 1 year when stored in the original containers at room temperature. To ensure stability, the bulk chemical was stored in the original metal cylinders at 10 ° to 22 ° C. Stability was monitored during the 13-week and 2-year studies using gas chromatography. The relative concentrations of perfluorocyclobutane were expected to increase due to the slow dimerization of tetrafluoroethylene; therefore, the gas cylinders were monitored for this dimer as well as for *d*-limonene during the 2-year studies. Two cylinders from lot 12438-6 contained higher concentrations of these compounds than canisters from the same lot that were analyzed earlier in the studies. One of these two cylinders, which contained 1.21% perfluorocyclobutane and 0.56% *d*-limonene, was replaced immediately after gas chromatographic analysis was performed; it is not known if the dimer formed in the canister due to degradation of tetrafluoroethylene or was present as an impurity at receipt. Stability was generally considered acceptable throughout the studies.

VAPOR GENERATION AND EXPOSURE SYSTEM

Because tetrafluoroethylene is a gas at room temperature, the generation and delivery system incorporated gas distribution under regulated pressure with individual adjustment and monitoring of the chemical flow

rate to each chamber. Tetrafluoroethylene was taken directly from the cylinder in which it was shipped and was metered to each exposure chamber. A sample line was included downstream from each gas cylinder. Stainless-steel chambers designed at Battelle Pacific Northwest Laboratories were used for all studies (Figure I3).

VAPOR CONCENTRATION MONITORING

The concentrations of tetrafluoroethylene were monitored using an on-line gas chromatograph, and samples were drawn and analyzed from each exposure chamber, the control chamber, the exposure suite, and an on-line standard. Summaries of the chamber concentrations during the studies are presented in Tables I1 through I3. The monthly mean exposure concentrations for the 2-year studies are presented in Figures I7 through I15; all were within 10% of the acceptable concentration range.

CHAMBER ATMOSPHERE CHARACTERIZATION

The time for the exposure concentration to build up to 90% of the final exposure concentration (T_{90}) used for all studies was 12 minutes. During the 16-day studies, T_{90} was approximately 12 minutes and T_{10} (the time for the exposure concentration to decay to 10% of the exposure concentration) was approximately 9 minutes with and without animals in the chambers. During the 13-week studies, T_{90} was 11 minutes with animals in the chambers and ranged from 11 to 14 minutes without animals; T_{10} ranged from 9 to 11 minutes with and without animals in the chambers. During the 2-year studies, T_{90} ranged from 8 to 10 minutes without animals in the chambers and from 10 to 16 minutes with animals present; T_{10} ranged from 7 to 9 minutes without animals and from 9 to 12 minutes with animals. The T_{90} value was not affected by the presence of animals.

Uniformity of tetrafluoroethylene concentration in the exposure chambers was measured using on-line gas chromatography before each of the studies, once during the 16-day and 13-week studies, and approximately every 3 months during the 2-year studies.

Uniformity of exposure concentrations within each chamber was acceptable, and relative standard deviations were less than 5%.

The persistence of tetrafluoroethylene in the 1,250 ppm exposure chamber after shutting off the system was monitored without animals before the 2-year studies began and with animals present during the 2-year studies. The concentration of tetrafluoroethylene in the exposure chamber fell rapidly to less than 1% of the beginning concentration within 20 minutes. Tetrafluoroethylene concentrations in the building exhaust and room air were also monitored during all studies.

Information supplied by the manufacturer indicated that all cylinders contained *d*-limonene as a stabilizer. *d*-Limonene and perfluorocyclobutane are less volatile than tetrafluoroethylene and concentrate in the cylinder as the tetrafluoroethylene is removed. Cylinder usage was regulated to minimize concentrations of these chemicals in the exposure chambers; a maximum of 80% of the tetrafluoroethylene in each cylinder was used. Gas chromatography of chamber samples and cylinder headspace in used and unused cylinders was conducted for *d*-limonene and perfluorocyclobutane. The results indicated that tetrafluoroethylene was stable during generation and in the exposure chambers and that perfluorocyclobutane was not formed in significant quantities by the generation system.

16-DAY STUDIES

Male and female F344/N rats and B6C3F₁ mice were obtained from Frederick Cancer Research Facility (Frederick, MD). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 11 days (rats) or 12 days (mice) and were approximately 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice were exposed to tetrafluoroethylene at concentrations of 0, 312, 625, 1,250, 2,500, and 5,000 ppm. The animals were exposed for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 12 exposure days during a 16-day period. Feed was available *ad libitum* except during exposure periods, and water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded two times during each exposure day for rats and mice. The animals were weighed on days 1, 8, and 15 and a t

necropsy. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 16-day studies, blood was collected from the retroorbital (rats) or supraorbital (mice) sinus for hematology analyses. Rats and mice were anesthetized with CO₂. Blood for hematology determinations was placed in tubes containing potassium EDTA as the anticoagulant. Analyses were performed with an Ortho ELT-8/ds hematology analyzer (Ortho Instruments; Westwood, MA). Leukocyte differential counts were determined by light microscopic examination of blood films stained with Wright-Giemsa. Reticulocyte counts were determined by light microscopy using smears stained supravivally with New Methylene Blue and a Miller disc for reticulocyte quantitation. Hematology parameters measured are listed in Table 1.

A necropsy was performed on all rats and mice. The brain, heart, left and right kidneys, liver, lung, right testis, and thymus were weighed. Histopathologic examinations were performed on all control and 5,000 ppm rats and mice. Selected organs were examined to a no-effect level in groups of animals exposed to lower concentrations of tetrafluoroethylene. Table 1 lists the tissues and organs examined.

13-WEEK STUDIES

The 13-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to tetrafluoroethylene and to determine the appropriate exposure levels to be used in the 2-year studies.

Male and female F344/N rats and B6C3F₁ mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, rats and mice were approximately 4 weeks old. Animals were quarantined for 12 days (rats) or 14 days (mice) and were approximately 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were exposed to tetrafluoroethylene at concentrations of 0, 312, 625, 1,250, 2,500, or 5,000 ppm. (One female rat assigned to the 2,500 ppm group was missexed and discarded at week 6.) The animals were exposed for 6 hours plus T₉₀ (12 minutes) per day, 5 days per week, for 13 weeks (excluding holidays). Feed was available *ad libitum* except during exposure periods, and water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded once weekly for rats and mice. The animals were weighed prior to the start of the studies and weekly thereafter. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 13-week studies, blood was collected from the retroorbital (rats) or supraorbital (mice) sinus for hematology analyses. Rats and mice were anesthetized with CO₂. Hematology determinations were analyzed as described for the 16-day study. Fourteen days (rats) or 10 days (mice) before the end of the studies, rats and mice were placed in metabolism cages (with access to water but not food) for a 16-hour urine collection. Four days before the end of the studies, rats were again placed in metabolism cages and were deprived of water for 16 hours to determine urine concentrating ability; urine was expressed from the bladders of all rats, and urine was collected for the following 4 hours while water deprivation continued. Specific gravity was determined on an A/O refractometer; urine glucose was determined using an Abbott VP glucose oxidase methodology (Abbott Laboratories, Abbott Park, IL); urine protein was determined by a Coomassie blue reaction; and fluoride was measured using an ion-specific electrode. Hematology and urinalysis parameters measured are listed in Table 1.

At the end of the 13-week studies, samples were collected for sperm morphology and vaginal cytology evaluations on rats and mice exposed to 0, 312, 1,250, and 5,000 ppm. The parameters evaluated are listed in Table 1. Methods used were those described in the NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1984). For 7 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells

were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm morphology, count, and motility. The right testis and right epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each right cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65 ° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

A necropsy was performed on all animals. The brain, heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all control and 5,000 ppm rats and mice and on selected organs from rats and mice exposed to lower concentrations. Table 1 lists the tissues and organs examined.

2-YEAR STUDIES

Study Design

Groups of 60 male rats were exposed to tetrafluoroethylene at concentrations of 0, 156, 312, and 625 ppm for 6 hours plus T₉₀ (12 minutes) per day, 5 days per week, for 104 weeks. Groups of 60 female rats and 58 male and female mice were exposed to tetrafluoroethylene at concentrations of 0, 312, 625, and 1,250 ppm for 6 hours plus T₉₀ (12 minutes) per day, 5 days per week, for 104 weeks (female rats) or

95 to 96 weeks (mice). Following the last day of exposure, rats were observed for approximately 11 days before necropsy. Ten male and ten female rats and mice from each group were evaluated at 15 months for alterations in hematology, clinical chemistry, and urinalysis parameters (rats only) and organ weights (rats and mice).

Source and Specification of Animals

Male and female F344/N rats and B6C3F₁ mice were obtained from Simonsen Laboratories (Gilroy, CA) for use in the 2-year studies. Rats and mice were quarantined for 15 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats and mice were housed individually. Feed was available *ad libitum* except during exposure periods, and water was available *ad libitum*. Chambers and cages were rotated weekly. Further details of animal maintenance are provided in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded monthly until the last 13 weeks (rats) or 5 weeks (mice), when they were recorded twice monthly. Animals were weighed prior to the start of the studies, weekly for the first 13 weeks, and monthly thereafter, until the last 13 weeks (rats) or 5 weeks (mice), when they were weighed every 2 weeks.

Ten male and ten female rats and mice per exposure group were designated for interim evaluation at 15 months. Two weeks before the scheduled evaluation, designated rats were placed in metabolism cages for a 16-hour urine collection. One week later, male and female rats were again placed in metabolism cages for a urine concentrating study. Rats were deprived of water for 16 hours, and urine was expressed from the bladders of the animals; urine was then collected during the next 6 hours for a urine concentrating study.

At the 15-month interim evaluation, blood was taken from the retroorbital plexus of rats for hematology and clinical chemistry analyses. The methods used were those described for the 13-week studies, and parameters measured are listed in Table 1.

A complete necropsy and microscopic examination were performed on all rats and mice. At the 15-month interim evaluation, the right kidney, liver, and lung were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, imbedded in paraffin, sectioned to a thickness of 5 to 6 μm , and stained with hematoxylin and eosin for microscopic examination. For all paired organs (i.e., adrenal gland, kidney, ovary), samples from each organ were examined. For extended evaluation of renal proliferative lesions, kidneys were step sectioned at 1 mm intervals, and six to ten additional sections were obtained from each kidney. 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 slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year rat and mouse studies, a quality assessment pathologist reviewed the following organs: adrenal medulla, kidney, liver, and spleen (male rats); eye, kidney, liver, and spleen (female rats); kidney, liver, and spleen (male mice); and harderian gland, liver, and spleen (female mice).

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment

pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contract research pathologists, the PWG chairperson, 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 Mc Connell *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, and D5 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, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the

denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm, i.e., the Kaplan-Meier estimate of the neoplasm incidence that would have been observed at the end of the study in the absence of mortality from all other competing risks (Kaplan and Meier, 1958).

Analysis of Neoplasm Incidences

For those neoplasms considered incidental in these studies, the primary statistical method used was a 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, a procedure based on the overall proportion of affected animals, was used.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoa data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973). Because the vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure levels.

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, which is updated yearly, are included in the NTP reports for neoplasms appearing to show compound-related effects.

QUALITY ASSURANCE METHODS

The 13-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a 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 tetrafluoroethylene was assessed by testing the ability of the chemical to induce increases in the frequency of micronucleated erythrocytes in peripheral blood samples obtained from male and female mice at the end of the 13-week study. The protocol for this study and the results are given in Appendix E.

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetrafluoroethylene

| 16-Day Studies | 13-Week Studies | 2-Year Studies |
|--|--|---|
| Study Laboratory | | |
| Battelle Pacific Northwest Laboratories (Richland, WA) | Battelle Pacific Northwest Laboratories (Richland, WA) | Battelle Pacific Northwest Laboratories (Richland, WA) |
| Strain and Species | | |
| Rats: F344/N Mice: B6C3F ₁ | Rats: F344/N Mice: B6C3F ₁ | Rats: F344/N Mice: B6C3F ₁ |
| Animal Source | | |
| Frederick Cancer Research Facility (Frederick, MD) | Taconic Farms, Inc. (Germantown, NY) | Simonsen Laboratories (Gilroy, CA) |
| Time Held Before Studies | | |
| Rats: 11 days Mice: 12 days | Rats: 12 days Mice: 14 days | 15 days |
| Average Age When Studies Began | | |
| 6 weeks | 6 weeks | 7 weeks |
| Date of First Exposure | | |
| Rats: 30 September 1985 Mice: 1 October 1985 | Rats: 25 March 1986 Mice: 27 March 1986 | Rats: 23 June 1988 Mice: 9 June 1988 |
| Duration of Exposure | | |
| 6 hours plus T ₉₀ (12 minutes) per day, 5 days per week, for 16 days | 6 hours plus T ₉₀ (12 minutes) per day, 5 days per week, for 13 weeks | 6 hours plus T ₉₀ (12 minutes) per day, 5 days per week, for 104 weeks (rats) (followed by an observation period of approximately 11 days), or 95 to 96 weeks (mice) |
| Date of Last Exposure | | |
| Rats: 15 October 1985 Mice: 16 October 1985 | Rats: 24 June 1986 (males) 25 June 1986 (females) Mice: 26 June 1986 (males) 25 June 1985 (females) | Rats: 15-month interim evaluation 17-18 September 1989 terminal sacrifice 14 June 1990 Mice: 15-month interim evaluation 6-7 September 1989 terminal sacrifice 4-5 April 1990 |
| Necropsy Dates | | |
| Rats: 16 October 1985 Mice: 17 October 1985 | Rats: 24 June 1986 (males) 25 June 1986 (females) Mice: 27 June 1986 (males) 26 June 1986 (females) | Rats: 15-month interim sacrifice 18-19 September 1989 terminal sacrifice 25 June 1990 (males) or 26-27 June 1990 (females) Mice: 15-month interim sacrifice 7-8 September 1989 terminal sacrifice 5 April 1990 (males) or 6 April 1990 (females) |

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetrafluoroethylene (continued)

| 16-Day Studies | 13-Week Studies | 2-Year Studies |
|---|---|--|
| Average Age at Necropsy 8 weeks | 19 weeks | Rats: 15-month interim evaluation - 71 weeks terminal sacrifice - 111-112 weeks Mice: 15-month interim evaluation - 72 weeks terminal sacrifice - 102 weeks |
| Size of Study Groups 5 males and 5 females | 10 males and 10 females (one rat assigned to the 2,500 ppm female group was determined to be missexed and was discarded during week 6) | 15-Month interim evaluation: 10 males and 10 females Terminal sacrifice: 50 males and 50 females (rats) or 48 males and 48 females (mice) |
| Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weight. | Same as 16-day studies | Same as 16-day studies |
| Animals per Cage 1 | 1 | 1 |
| Method of Animal Identification Toe clips and placement within cage unit | Same as 16-day studies | Rats: Tail tattoos and placement within cage unit Mice: Toe clips and placement within cage unit |
| Diet NIH-07 pelleted feed (Zeigler Brothers, Inc., Gardners, PA); available <i>ad libitum</i> , except during exposure periods | Same as 16-day studies | Same as 16-day studies |
| Maximum Storage Time for Feed 120 days post-milling | Same as 16-day studies | Same as 16-day studies |
| Water Tap water (Richland municipal supply) via automatic watering system (Edstrom Industries; Waterford, WI); available <i>ad libitum</i> | Same as 16-day studies | Same as 16-day studies |
| Cages Stainless steel (Hazleton Systems, Inc., Aberdeen, MD), changed weekly | Same as 16-day studies | Stainless steel (Lab Products, Inc., Harford Division, Aberdeen, Md), changed weekly |
| Bedding/Cage Board Techsorb®, Shepherd Specialty Papers, Inc. (Kalamazoo, MI), used during non-exposure periods, 7 days per week | Same as 16-day studies | Same as 16-day studies |

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetrafluoroethylene (continued)

| 16-Day Studies | 13-Week Studies | 2-Year Studies |
|--|---|---|
| <p>Chamber Air Supply Filters Duct pre-filter, dual and single HEPA (American Air Filter, Louisville, KY); checked biannually</p> | Same as 16-day studies | Single HEPA (Flanders Filters, Inc., San Rafael, CA) and charcoal (RSE, Inc., New Baltimore, MI); changed before the studies began and once yearly thereafter |
| <p>Chambers Stainless steel (Hazleton Systems, Inc., Aberdeen, MD); changed weekly</p> | Stainless steel (Hazleton Systems, Inc., Aberdeen, MD); changed weekly | Stainless steel (Lab Products, Inc., Harford Division, Aberdeen, MD); changed weekly |
| <p>Chamber Environment Temperature: 22.3° – 23.2° C Relative humidity: 52.7% – 55.4% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour</p> | <p>Temperature: 23.1° – 23.7° C Relative humidity: 56.1% – 60.5% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour</p> | <p>Temperature: 24.1° – 24.5° C Relative humidity: 53% – 58% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour</p> |
| <p>Exposure Concentrations 0, 312, 625, 1,250, 2,500, or 5,000 ppm</p> | 0, 312, 625, 1,250, 2,500, or 5,000 ppm | <p>Rats: males - 0, 156, 312, or 625 ppm females - 0, 312, 625, or 1,250 ppm Mice: 0, 312, 625, or 1,250 ppm</p> |
| <p>Type and Frequency of Observation All animals were observed twice daily. Clinical findings were recorded two times each exposure day. All animals were weighed on days 1, 8, 15, and at necropsy.</p> | <p>All animals were observed twice daily. Clinical findings were recorded once weekly for rats and mice. The animals were weighed before the studies began and weekly thereafter.</p> | <p>All animals were observed twice daily. Clinical findings were recorded monthly until the last 13 weeks (rats) or 5 weeks (mice), when they were recorded twice monthly. Animals were weighed before the studies began, weekly for the first 13 weeks, and monthly thereafter, until the last 13 weeks (rats) or 5 weeks (mice) when they were weighed every 2 weeks.</p> |
| <p>Method of Sacrifice Anesthetization with sodium pentobarbital followed by exsanguination</p> | Anesthetization with CO ₂ followed by exsanguination | Anesthetization with CO ₂ followed by exsanguination |
| <p>Necropsy Necropsy performed on all animals. Organs weighed were brain, heart, left and right kidneys, liver, lung, right testis, and thymus.</p> | <p>Necropsy performed on all animals. Organs weighed were brain, heart, right kidney, liver, lung, right testis, and thymus.</p> | <p>Necropsy performed on all animals. Organs weighed at the 15-month interim evaluation were the right kidney, liver, and lung.</p> |

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetrafluoroethylene (continued)

| 16-Day Studies | 13-Week Studies | 2-Year Studies |
|---|---|---|
| <p>Clinical Pathology Blood was collected from all animals from the retroorbital (rats) or supraorbital (mice) sinus for hematology analyses. Hematology: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials</p> | <p>Blood was collected from all animals from the retroorbital (rats) or supraorbital (mice) sinus for hematology analyses. Fourteen days (rats) or 10 days (mice) before the end of the studies, animals were placed in metabolism cages (with access to water but not food) for a 16-hour urine collection. Four days before the end of the studies, rats were again placed in metabolism cages and were deprived of water for 16 hours; urine was expressed from the bladders of all rats, and urine was collected for the following 4 hours while water deprivation continued. Hematology: hematocrit; hemoglobin concentration; erythrocyte and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials Urinalysis: fluoride, glucose, and protein concentrations; volume; and specific gravity</p> | <p>At the 15-month interim evaluation, blood was taken from the retroorbital plexus of rats for hematology and clinical chemistry analyses. Two weeks prior to the scheduled evaluation, designated rats were placed in metabolism cages for a 16-hour urine collection. One week prior to the evaluation, rats were again placed in metabolism cages and were deprived of water for 16 hours; urine was expressed from the bladders of all rats, and urine was collected for the following 6 hours while water deprivation continued. Hematology: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials Clinical chemistry: urea nitrogen, creatinine, sodium, potassium, chloride, calcium, and phosphorus concentrations Urinalysis: glucose and protein concentrations; volume; and specific gravity</p> |
| <p>Histopathology Complete histopathology was performed on rats and mice exposed to 0 or 5,000 ppm. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland, esophagus, eye (if grossly abnormal), gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lungs, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, nose, ovaries, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, sternbrae (including marrow), stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. Additionally, the kidney of all other exposed groups of male and female rats and mice was examined, as was the pancreas of male and female rats exposed to 2,500 ppm.</p> | <p>Complete histopathology was performed on rats and mice exposed to 0 or 5,000 ppm. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland, esophagus, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lungs, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, nose, ovaries, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, sternbrae (including marrow), stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. Additionally, the following organs were examined in lower exposure groups: the kidney of all exposed groups of male and female rats and mice (except female rats exposed to 312 ppm); the cecum of 625 ppm male rats; the eye and mandibular lymph node of 2,500 ppm female rats; and the thymus and lung of 625 ppm male mice.</p> | <p>Complete histopathology was performed on all control and exposed rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland, esophagus, femur, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lungs, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, muscle (thigh), nose, oral cavity (larynx and pharynx), ovaries, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, sternbrae (including marrow), stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.</p> |

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetrafluoroethylene (continued)

| 16-Day Studies | 13-Week Studies | 2-Year Studies |
|--|--|----------------|
| Sperm Morphology and Vaginal Cytology None | At terminal sacrifice, sperm samples were collected from all male animals in the 0, 312, 1,250, and 5,000 ppm groups for sperm morphology evaluations. The parameters evaluated included sperm morphology, concentration, and motility. The right cauda, right epididymis, and right testis were weighed. Vaginal samples were collected for up to 7 consecutive days prior to the end of the studies from all females exposed to 0, 312, 1,250, or 5,000 ppm for vaginal cytology evaluations. The parameters evaluated included the relative frequency of estrous stages and estrous cycle length. | None |

RESULTS

RATS

16-DAY STUDY

All rats survived to the end of the study (Table 2). The final mean body weights and body weight gains of males and females exposed to 5,000 ppm were significantly less than those of the controls. The mean body weight gain of females exposed to 2,500 ppm was also significantly less than that of the control group. There were no exposure-related clinical findings in male or female rats.

There were no significant differences in hematology parameters that were considered to be related to

tetrafluoroethylene exposure (Table G1). Absolute and relative left and right kidney weights of all exposed groups of males were significantly greater than those of the controls (Table F1), as were those of females in the 2,500 and 5,000 ppm groups. The absolute left and right kidney weight of females exposed to 1,250 ppm was also significantly greater than that of the controls. All exposed groups of males had significantly greater relative liver weights than the controls; males in the 625 and 2,500 ppm groups also had greater absolute liver weights than the controls. Other organ weight differences were not considered to be related to chemical exposure.

TABLE 2
Survival and Body Weights of Rats in the 16-Day Inhalation Study of Tetrafluoroethylene

| Exposure Concentration (ppm) | Survival ^a | Mean Body Weight ^b (g) | | | Final Weight Relative to Controls (%) |
|------------------------------|-----------------------|-----------------------------------|-----------|----------|---------------------------------------|
| | | Initial | Final | Change | |
| Male | | | | | |
| 0 | 5/5 | 120 ± 3 | 167 ± 4 | 48 ± 1 | |
| 312 | 5/5 | 121 ± 2 | 176 ± 4 | 55 ± 3 | 105 |
| 625 | 5/5 | 122 ± 1 | 172 ± 3 | 50 ± 3 | 103 |
| 1,250 | 5/5 | 119 ± 1 | 165 ± 1 | 46 ± 1 | 99 |
| 2,500 | 5/5 | 119 ± 1 | 162 ± 2 | 43 ± 2 | 96 |
| 5,000 | 5/5 | 116 ± 2 | 143 ± 3** | 27 ± 2** | 86 |
| Female | | | | | |
| 0 | 5/5 | 98 ± 1 | 126 ± 3 | 28 ± 2 | |
| 312 | 5/5 | 104 ± 2* | 131 ± 2 | 27 ± 2 | 104 |
| 625 | 5/5 | 97 ± 2 | 125 ± 3 | 28 ± 2 | 99 |
| 1,250 | 5/5 | 103 ± 1 | 131 ± 3 | 28 ± 3 | 104 |
| 2,500 | 5/5 | 100 ± 2 | 119 ± 4 | 19 ± 3* | 94 |
| 5,000 | 5/5 | 99 ± 1 | 113 ± 2* | 14 ± 1** | 89 |

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

** $P \leq 0.01$

^a Number of animals surviving at 16 days/number initially in group

^b Weights and weight changes are given as mean ± standard error.

Increased incidences of renal tubule degeneration occurred in males and females exposed to 625 ppm or greater (Table 3). The severity of these lesions increased with increasing exposure concentration and was slightly greater in males than in females. Renal lesions occurred predominantly in the inner cortical tubular epithelial cells at the junction of the cortex and medulla. Degeneration was characterized by inner cortical tubules that contained luminal debris and were lined by cells with vacuolated or eosinophilic cytoplasm. Other tubule epithelial cells had occasional dark pyknotic nuclei and shrunken, eosinophilic cytoplasm. Associated with renal tubule degeneration was regeneration of renal cortical tubule

epithelium, characterized by epithelial cells with basophilic cytoplasm, enlarged vesicular nuclei, and slightly increased numbers of mitotic figures. The increased kidney weights were associated with the renal lesions. Histopathologic examination revealed no chemical-related lesions in the livers of exposed male or female rats.

Exposure Concentration Selection Rationale: Based on the lack of overt toxicity following exposure of male and female rats to 5,000 ppm for 16 days, the 13-week exposure concentrations selected were 0, 312, 625, 1,250, 2,500, and 5,000 ppm.

TABLE 3
Incidences of Nonneoplastic Lesions of the Kidney in Rats in the 16-Day Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|-------|---------|------------------------|-----------|-----------|-----------|
| Male | | | | | | |
| Number Examined Microscopically | 5 | 5 | 5 | 5 | 5 | 5 |
| Renal Tubule, Degeneration ^a | 0 | 0 | 5** (1.0) ^b | 5** (1.4) | 5** (2.0) | 5** (3.0) |
| Female | | | | | | |
| Number Examined Microscopically | 5 | 5 | 5 | 5 | 5 | 5 |
| Renal Tubule, Degeneration | 0 | 0 | 3 (1.0) | 5** (1.2) | 5** (1.8) | 5** (3.2) |

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test

^a Number of animals with lesion

^b Average severity grade of lesions in affected rats: 1=minimal; 2=mild; 3=moderate; 4=marked

13-WEEK STUDY

All rats survived to the end of the study (Table 4); one rat in the 2,500 ppm female group was missexed and was removed from the study. The final mean body weight and body weight gain of males and the mean body weight gain of females exposed to 5,000 ppm were significantly less than those of the controls. There were no clinical findings attributed to exposure to tetrafluoroethylene.

The hematology and clinical chemistry data for rats in the 13-week study are presented in Table G2. Exposure to tetrafluoroethylene caused a concentration-dependent anemia, evidenced by decreased hematocrit values, hemoglobin concentrations, and erythrocyte counts in all exposed groups of males and in females exposed to 5,000 ppm. There were no changes in the mean cell volume values of exposed groups of rats, indicating the erythrocytes were normocytic. There

were minimal decreases of the mean cell hemoglobin concentration in males exposed to 1,250 ppm or greater; this did not occur in exposed female rats. Decreases in mean cell hemoglobin concentration without alterations in mean cell volumes have been related to the early stages of iron deficiency. Because the mean cell hemoglobin concentration values of exposed groups of male rats were well within a normal physiological range and no changes occurred in exposed groups of females, the significance of the mean cell hemoglobin concentration alterations was considered questionable and the erythrocytes were characterized as normochromic. There were no increases in reticulocyte counts to indicate a bone marrow response to the anemia. Therefore, the anemia would be characterized as normocytic, normochromic, and nonresponsive. Normocytic, normochromic, nonresponsive anemias have been related to selective suppression of erythropoiesis in a variety of disorders and may be due to decreased erythropoietin

TABLE 4
Survival and Body Weights of Rats in the 13-Week Inhalation Study of Tetrafluoroethylene

| Exposure Concentration (ppm) | Survival ^a | Mean Body Weight ^b (g) | | | Final Weight Relative to Controls (%) |
|------------------------------|-----------------------|-----------------------------------|-----------|-----------|---------------------------------------|
| | | Initial | Final | Change | |
| Male | | | | | |
| 0 | 10/10 | 116 ± 3 | 328 ± 5 | 212 ± 3 | |
| 312 | 10/10 | 117 ± 2 | 337 ± 5 | 220 ± 4 | 103 |
| 625 | 10/10 | 115 ± 2 | 339 ± 4 | 225 ± 3 | 103 |
| 1,250 | 10/10 | 114 ± 2 | 330 ± 4 | 216 ± 4 | 101 |
| 2,500 | 10/10 | 113 ± 2 | 327 ± 4 | 214 ± 4 | 100 |
| 5,000 | 10/10 | 115 ± 3 | 299 ± 6** | 184 ± 4** | 91 |
| Female | | | | | |
| 0 | 10/10 | 91 ± 2 | 185 ± 6 | 94 ± 4 | |
| 312 | 10/10 | 97 ± 3 | 196 ± 3 | 99 ± 3 | 106 |
| 625 | 10/10 | 93 ± 2 | 195 ± 5 | 102 ± 5 | 105 |
| 1,250 | 10/10 | 95 ± 2 | 192 ± 4 | 97 ± 3 | 104 |
| 2,500 | 9/9 | 89 ± 4 | 177 ± 7 | 88 ± 4 | 95 |
| 5,000 | 10/10 | 94 ± 2 | 174 ± 4 | 80 ± 3* | 94 |

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

** $P \leq 0.01$

^a Number of animals surviving at 13 weeks/number initially in group

^b Weights and weight changes are given as mean ± standard error.

elaboration, bone marrow suppression, or defective iron metabolism.

A concentration-dependent proteinuria occurred in all exposed groups of male rats and in females exposed to 2,500 or 5,000 ppm. Proteinuria could be related to glomerular or renal tubule injury and is consistent with the renal tubule degeneration observed histopathologically. However, the renal injury did not appear to affect the ability of the kidney to concentrate the urine, as evidenced by appropriate increases in urine specific gravity in response to a water concentration test. There was a concentration-dependent increase of urinary fluoride excretion in all exposed groups of male and female rats, consistent with metabolism and excretion of tetrafluoroethylene. A possible polyuria, evidenced by increased 16-hour urine volumes, occurred in males in the 5,000 ppm group and in all exposed groups of females.

The absolute and relative liver weights of all exposed groups of males and of females in the 5,000 ppm group were significantly greater than those of the controls (Table F2). The absolute and relative right kidney weights of males and females exposed to 1,250 ppm or greater and of females in the 625 ppm

group were also significantly greater than those of the controls. The absolute and relative heart weights of males exposed to 1,250, 2,500, or 5,000 ppm were generally significantly greater than those of the controls. Other organ weight differences were not considered to be related to chemical exposure.

There were no differences in sperm morphology parameters or in the length of estrous cycles between control and exposed groups of rats (Tables H1 and H2).

Incidences of renal tubule degeneration in males exposed to 625 ppm or greater and in females exposed to 2,500 or 5,000 ppm were significantly greater than those in the controls (Table 5). The renal lesions, which were similar to those observed in the 16-day study, increased in severity with exposure concentration and were slightly more severe in males than females. Renal lesions were observed at the junction of the cortex and medulla and involved inner cortical tubules. Degeneration was characterized by inner cortical tubules that were dilated, contained luminal debris, and had occasional pyknotic nuclei. Associated with degeneration was regeneration, characterized by renal tubule epithelial cells that were lined by cells with basophilic cytoplasm having pleomorphic

TABLE 5
Incidences of Nonneoplastic Lesions of the Kidney in Rats in the 13-Week Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|-------|----------------|-------------------------|------------|------------|------------|
| Male | | | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 | 10 | 10 |
| Renal Tubule, Degeneration ^a | 0 | 0 | 10** (1.0) ^b | 10** (1.0) | 10** (2.0) | 10** (2.0) |
| Female | | | | | | |
| Number Examined Microscopically | 10 | — ^c | 10 | 10 | 9 | 10 |
| Renal Tubule, Degeneration | 0 | | 0 | 0 | 9** (1.0) | 10** (2.0) |

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test

^a Number of animals with lesion

^b Average severity grade of lesions in affected rats: 1=minimal; 2=mild; 3=moderate; 4=marked

^c Kidney not examined at this exposure concentration

nuclei with large, prominent nucleoli, a slightly increased number of mitotic figures, and varying degrees of karyomegaly. The increased kidney weights were associated with the renal lesions. Histopathologically, there were no chemical-related lesions in the livers of male or female rats exposed to tetrafluoroethylene.

Exposure Concentration Selection Rationale: Exposure concentrations for the 2-year rat study in males were based on kidney effects. Although the severity and incidence of kidney lesions were identical for male rats exposed to 625 or 1,250 ppm, the absolute and relative kidney weights of males exposed to 1,250 ppm were significantly greater than those of the controls. Therefore, the highest exposure concentration selected was 625 ppm, which was expected to cause minimal toxicity in the kidney following 2 years of exposure.

Although kidney effects were the main toxicologic consideration for exposure concentration selection in the 2-year study in female rats, the highest exposure

concentration was selected, in part, based on the number of chambers to be used in this inhalation study. When possible in inhalation studies, males and females of a species for each concentration are housed in the same chamber. When it is obvious that the same exposure concentration will cause more severe effects in one sex of the same species, space is sought in chambers housing the other species. In the present study, concentrations selected for the 2-year mouse study were 312, 625, and 1,250 ppm. At the time of selection of concentrations for the mouse study, it was believed that female rats could tolerate exposure to 2,500 ppm with minimal toxic effects. However, it was decided that an additional chamber solely for female rats could not be justified economically; therefore, female rats were housed in the same chambers as mice. In addition, because of the increased absolute and relative kidney weights of females exposed to 625 ppm or greater (even in the absence of histopathologic effects), it was believed that toxicity might occur at 625 or 1,250 ppm. Therefore, because there was additional space in the mouse chambers, 1,250 ppm was selected as the highest exposure concentration for the 2-year study in female rats.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan - Meier survival curves (Figure 1). The survival rates of males in the 625 ppm group and of all exposed groups of females were significantly less than those of the controls.

Body Weights and Clinical Findings

Mean body weights of males exposed to 625 ppm were lower than those of the controls from week 81 until the end of the study; the mean body weights of males exposed to 156 or 312 ppm and of females exposed to 312 or 625 ppm were similar to those of

the controls throughout the study (Figure 2 and Tables 7 and 8). Mean body weights of females exposed to 1,250 ppm were slightly lower than those of the controls at the end of the study; the final mean body weight of this group was 91% that of the controls. The only clinical finding associated with exposure to tetrafluoroethylene was opacity of one or both eyes in exposed groups of female rats; this change was observed microscopically as cataracts.

Hematology, Clinical Chemistry, and Urinalysis

At the 15-month interim evaluation, no differences in hematology, clinical chemistry, or urinalysis parameters were considered to be related to tetrafluoroethylene exposure (Table G3).

TABLE 6
Survival of Rats in the 2-Year Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--|---------|-----------------|---------|-----------|
| Male | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation ^a | 10 | 10 | 10 | 10 |
| Moribund | 27 | 34 | 32 | 48 |
| Natural deaths | 6 | 4 | 1 | 1 |
| Animals surviving to study termination | 17 | 12 | 17 | 1 |
| Percent probability of survival at end of study ^b | 34 | 24 | 34 | 2 |
| Mean survival (days) ^c | 665 | 644 | 665 | 609 |
| Survival analyses ^d | P<0.001 | P=0.216 | P=0.829 | P<0.001 |
| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
| Female | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation ^a | 10 | 10 | 10 | 10 |
| Accidental death ^a | 0 | 0 | 0 | 1 |
| Moribund | 21 | 33 | 31 | 25 |
| Natural deaths | 1 | 1 | 4 | 6 |
| Animals surviving to study termination | 28 | 16 ^e | 15 | 18 |
| Percent probability of survival at end of study | 56 | 32 | 30 | 37 |
| Mean survival (days) | 694 | 642 | 651 | 620 |
| Survival analyses | P=0.034 | P=0.008 | P=0.007 | P=0.014 |

^a Censored from survival analyses

^b Kaplan-Meier determinations

^c Mean of all deaths (uncensored, censored, and terminal sacrifice)

^d The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns.

^e Includes one animal that died during the last week of the study

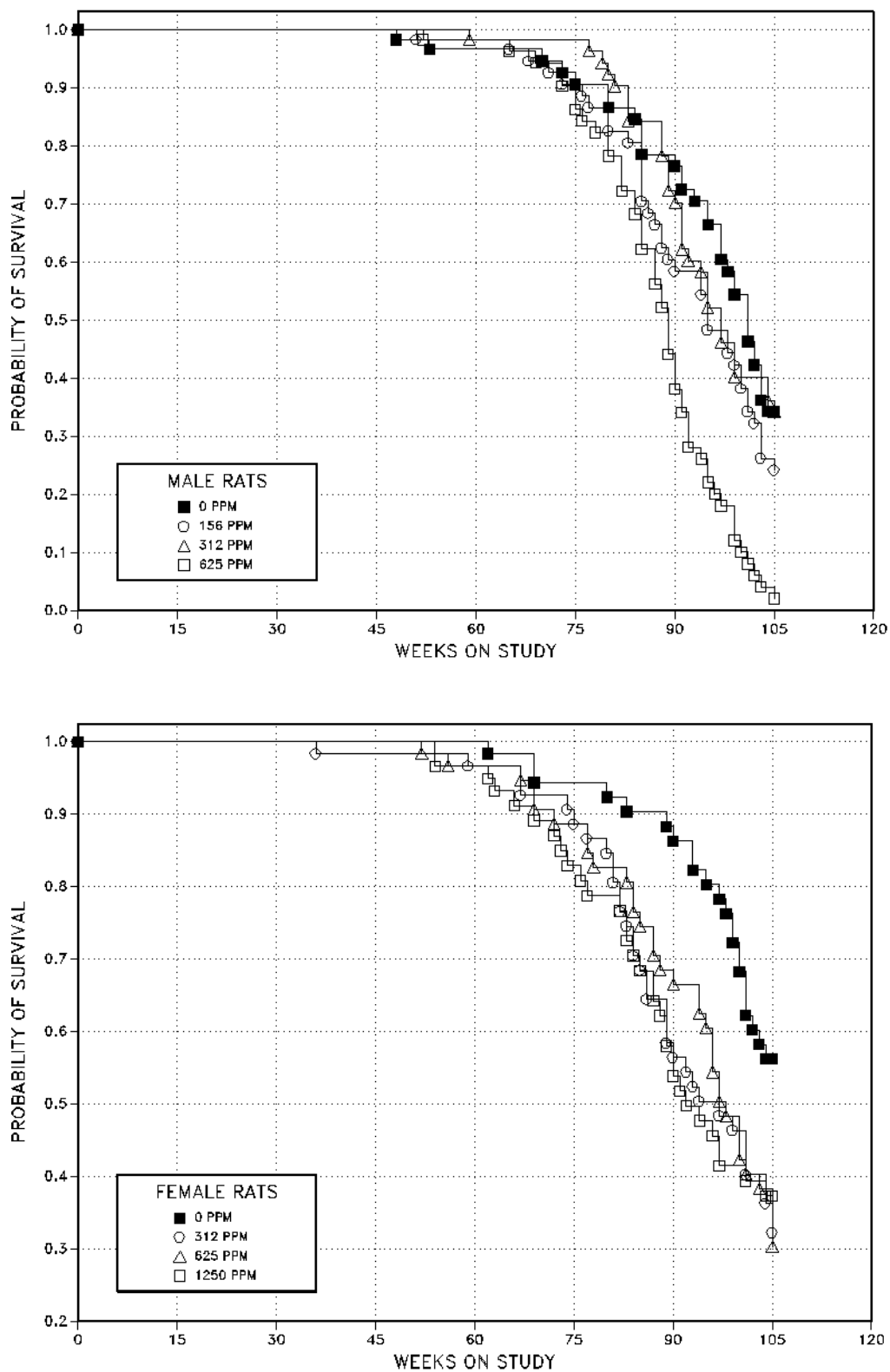


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Administered Tetrafluoroethylene by Inhalation for 2 Years

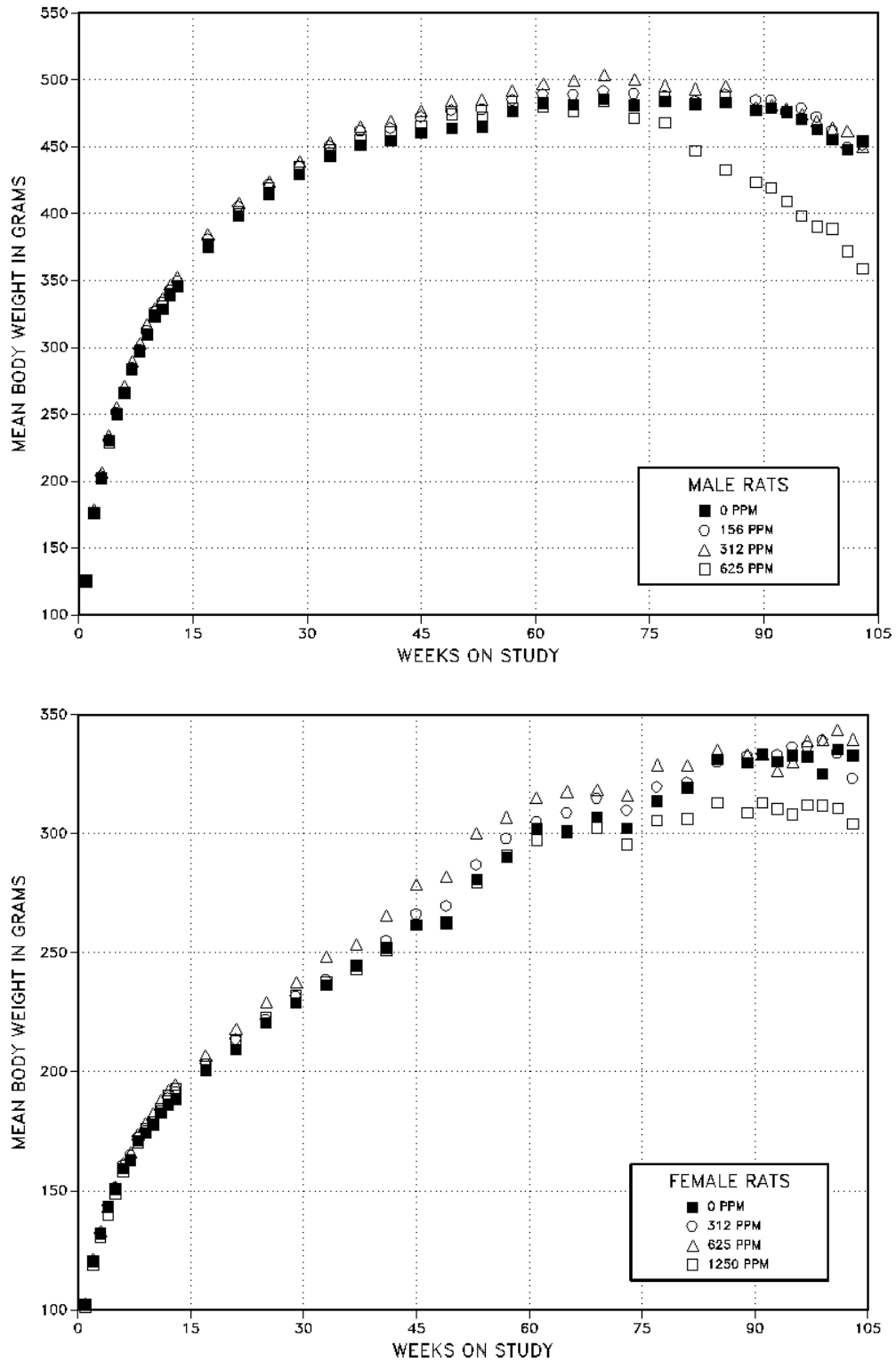


FIGURE 2
Growth Curves for Male and Female Rats Administered Tetrafluoroethylene by Inhalation for 2 Years

TABLE 7
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Tetrafluoroethylene

| Weeks on Study | 0 ppm | | 156 ppm | | | 312 ppm | | | 625 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 | 126 | 60 | 126 | 100 | 60 | 126 | 100 | 60 | 125 | 99 | 60 |
| 2 | 176 | 60 | 177 | 101 | 60 | 179 | 102 | 60 | 176 | 100 | 60 |
| 3 | 203 | 60 | 204 | 101 | 60 | 207 | 102 | 60 | 202 | 100 | 60 |
| 4 | 230 | 60 | 231 | 100 | 60 | 234 | 102 | 60 | 229 | 100 | 60 |
| 5 | 250 | 60 | 251 | 100 | 60 | 255 | 102 | 60 | 250 | 100 | 60 |
| 6 | 267 | 60 | 267 | 100 | 60 | 271 | 102 | 60 | 266 | 100 | 60 |
| 7 | 284 | 60 | 285 | 100 | 60 | 289 | 102 | 60 | 283 | 100 | 60 |
| 8 | 298 | 60 | 299 | 100 | 60 | 303 | 102 | 60 | 297 | 100 | 60 |
| 9 | 310 | 60 | 312 | 101 | 60 | 317 | 102 | 60 | 310 | 100 | 60 |
| 10 | 324 | 60 | 327 | 101 | 60 | 329 | 102 | 60 | 323 | 100 | 60 |
| 11 | 329 | 60 | 334 | 102 | 60 | 336 | 102 | 60 | 331 | 101 | 60 |
| 12 | 339 | 60 | 343 | 101 | 60 | 347 | 102 | 60 | 340 | 100 | 60 |
| 13 | 346 | 60 | 350 | 101 | 60 | 353 | 102 | 60 | 346 | 100 | 60 |
| 17 | 375 | 60 | 381 | 102 | 60 | 384 | 103 | 60 | 377 | 101 | 60 |
| 21 | 398 | 60 | 406 | 102 | 60 | 408 | 103 | 60 | 402 | 101 | 60 |
| 25 | 415 | 60 | 422 | 102 | 60 | 424 | 102 | 60 | 420 | 101 | 60 |
| 29 | 430 | 60 | 436 | 101 | 60 | 439 | 102 | 60 | 436 | 101 | 60 |
| 33 | 443 | 60 | 451 | 102 | 60 | 454 | 102 | 60 | 448 | 101 | 60 |
| 37 | 451 | 60 | 462 | 102 | 60 | 465 | 103 | 60 | 456 | 101 | 60 |
| 41 | 455 | 60 | 464 | 102 | 60 | 469 | 103 | 60 | 461 | 101 | 60 |
| 45 | 461 | 60 | 473 | 103 | 60 | 477 | 104 | 60 | 465 | 101 | 60 |
| 49 | 464 | 59 | 478 | 103 | 60 | 484 | 104 | 60 | 475 | 102 | 60 |
| 53 | 465 | 59 | 478 | 103 | 59 | 485 | 104 | 60 | 472 | 101 | 59 |
| 57 | 476 | 58 | 485 | 102 | 59 | 492 | 103 | 60 | 479 | 101 | 59 |
| 61 | 483 | 58 | 489 | 101 | 59 | 497 | 103 | 59 | 480 | 99 | 59 |
| 65 ^a | 482 | 48 | 489 | 102 | 48 | 499 | 104 | 49 | 477 | 99 | 49 |
| 69 | 486 | 48 | 492 | 101 | 47 | 504 | 104 | 49 | 484 | 100 | 47 |
| 73 | 481 | 47 | 490 | 102 | 45 | 501 | 104 | 49 | 472 | 98 | 47 |
| 77 | 484 | 45 | 488 | 101 | 43 | 496 | 102 | 49 | 468 | 97 | 42 |
| 81 | 482 | 43 | 485 | 101 | 41 | 493 | 102 | 45 | 447 | 93 | 39 |
| 85 | 483 | 41 | 490 | 101 | 38 | 495 | 103 | 42 | 433 | 90 | 33 |
| 89 | 477 | 39 | 485 | 102 | 30 | 479 | 100 | 36 | 423 | 89 | 22 |
| 91 | 479 | 36 | 485 | 101 | 29 | 481 | 101 | 31 | 419 | 88 | 17 |
| 93 | 476 | 35 | 477 | 100 | 29 | 478 | 101 | 30 | 409 | 86 | 14 |
| 95 | 471 | 34 | 479 | 102 | 26 | 475 | 101 | 28 | 399 | 85 | 11 |
| 97 | 463 | 31 | 473 | 102 | 24 | 469 | 101 | 23 | 390 | 84 | 9 |
| 99 | 456 | 27 | 462 | 102 | 21 | 464 | 102 | 21 | 389 | 85 | 6 |
| 101 | 448 | 24 | 449 | 100 | 17 | 462 | 103 | 20 | 372 | 83 | 4 |
| 103 | 454 | 19 | 452 | 99 | 13 | 450 | 99 | 20 | 359 | 79 | 3 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 268 | | 270 | 101 | | 273 | 102 | | 268 | 100 | |
| 14-52 | 432 | | 441 | 102 | | 445 | 103 | | 438 | 101 | |
| 53-103 | 473 | | 479 | 101 | | 484 | 102 | | 434 | 92 | |

^a Interim evaluation occurred during week 65.

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of Tetrafluoroethylene

| Weeks on Study | 0 ppm | | 312 ppm | | | 625 ppm | | | 1,250 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 | 102 | 60 | 102 | 100 | 60 | 102 | 100 | 60 | 101 | 99 | 60 |
| 2 | 121 | 60 | 121 | 100 | 60 | 121 | 101 | 60 | 119 | 99 | 59 |
| 3 | 132 | 60 | 132 | 100 | 60 | 133 | 101 | 60 | 130 | 99 | 59 |
| 4 | 143 | 60 | 144 | 100 | 60 | 143 | 100 | 60 | 140 | 98 | 59 |
| 5 | 151 | 60 | 151 | 101 | 60 | 152 | 101 | 60 | 149 | 99 | 59 |
| 6 | 159 | 60 | 161 | 101 | 60 | 161 | 101 | 60 | 158 | 99 | 59 |
| 7 | 163 | 60 | 165 | 101 | 60 | 166 | 102 | 60 | 163 | 100 | 59 |
| 8 | 171 | 60 | 173 | 101 | 60 | 174 | 101 | 60 | 170 | 100 | 59 |
| 9 | 174 | 60 | 175 | 101 | 60 | 178 | 102 | 60 | 176 | 101 | 59 |
| 10 | 178 | 60 | 179 | 101 | 60 | 183 | 103 | 60 | 179 | 101 | 59 |
| 11 | 183 | 60 | 184 | 101 | 60 | 188 | 103 | 60 | 185 | 101 | 59 |
| 12 | 186 | 60 | 189 | 102 | 60 | 192 | 103 | 60 | 190 | 102 | 59 |
| 13 | 189 | 60 | 192 | 102 | 60 | 195 | 103 | 60 | 193 | 102 | 59 |
| 17 | 201 | 60 | 203 | 101 | 60 | 207 | 103 | 60 | 203 | 101 | 59 |
| 21 | 209 | 60 | 214 | 102 | 60 | 218 | 104 | 60 | 213 | 102 | 59 |
| 25 | 220 | 60 | 222 | 101 | 60 | 229 | 104 | 60 | 223 | 101 | 59 |
| 29 | 229 | 60 | 232 | 101 | 60 | 238 | 104 | 60 | 232 | 101 | 59 |
| 33 | 236 | 60 | 239 | 101 | 60 | 248 | 105 | 60 | 238 | 101 | 59 |
| 37 | 245 | 60 | 245 | 100 | 59 | 253 | 104 | 60 | 243 | 99 | 59 |
| 41 | 252 | 60 | 255 | 101 | 59 | 265 | 105 | 60 | 251 | 100 | 59 |
| 45 | 262 | 60 | 266 | 102 | 59 | 279 | 106 | 60 | 262 | 100 | 59 |
| 49 | 263 | 60 | 270 | 103 | 59 | 282 | 107 | 60 | 262 | 100 | 59 |
| 53 | 281 | 60 | 287 | 102 | 59 | 300 | 107 | 59 | 279 | 100 | 59 |
| 57 | 290 | 60 | 298 | 103 | 59 | 307 | 106 | 58 | 291 | 100 | 57 |
| 61 | 302 | 60 | 305 | 101 | 58 | 315 | 104 | 58 | 297 | 98 | 57 |
| 65 ^a | 301 | 59 | 309 | 103 | 58 | 318 | 106 | 58 | 300 | 100 | 55 |
| 69 | 307 | 48 | 315 | 103 | 46 | 318 | 104 | 45 | 303 | 99 | 43 |
| 73 | 302 | 47 | 310 | 103 | 46 | 316 | 105 | 44 | 296 | 98 | 42 |
| 77 | 314 | 47 | 320 | 102 | 44 | 329 | 105 | 42 | 305 | 97 | 39 |
| 81 | 319 | 46 | 322 | 101 | 40 | 328 | 103 | 41 | 306 | 96 | 38 |
| 85 | 331 | 45 | 330 | 100 | 35 | 335 | 101 | 37 | 313 | 95 | 34 |
| 89 | 330 | 44 | 333 | 101 | 29 | 333 | 101 | 34 | 309 | 94 | 28 |
| 91 | 333 | 43 | 333 | 100 | 28 | 333 | 100 | 33 | 313 | 94 | 25 |
| 93 | 330 | 41 | 333 | 101 | 26 | 326 | 99 | 33 | 310 | 94 | 24 |
| 95 | 333 | 40 | 336 | 101 | 25 | 330 | 99 | 31 | 308 | 93 | 23 |
| 97 | 332 | 39 | 337 | 101 | 24 | 339 | 102 | 25 | 312 | 94 | 21 |
| 99 | 325 | 38 | 339 | 104 | 23 | 339 | 104 | 24 | 312 | 96 | 20 |
| 101 | 336 | 33 | 334 | 100 | 21 | 344 | 102 | 20 | 311 | 93 | 19 |
| 103 | 333 | 30 | 323 | 97 | 20 | 340 | 102 | 19 | 304 | 91 | 19 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 158 | | 159 | 101 | | 161 | 102 | | 158 | 100 | |
| 14-52 | 235 | | 238 | 101 | | 247 | 105 | | 236 | 100 | |
| 53-103 | 318 | | 321 | 101 | | 326 | 103 | | 304 | 96 | |

^a Interim evaluation occurred during week 65.

Pathology and Statistical Analyses

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

Absolute and relative lung weights of exposed groups of rats were similar to those of the controls at the 15-month interim evaluation (Table F3), and no lung lesions related to tetrafluoroethylene exposure occurred.

Kidney: At the 15-month interim evaluation, the absolute and relative right kidney weights of males exposed to 625 ppm and females exposed to 1,250 ppm were significantly greater than those of the controls (Table F3). Additionally, the absolute kidney weight of females exposed to 625 ppm and the relative kidney weight of males exposed to 312 ppm were significantly greater than those of the controls.

At 15 months, renal tubule hyperplasia was observed in one male exposed to 312 ppm and in one male exposed to 625 ppm. At the end of the study, the incidences of renal tubule hyperplasia in males exposed to 625 ppm and of renal tubule adenoma in males exposed to 312 ppm were significantly greater than those in the controls (Tables 9, A3, and A5). The combined incidence of renal tubule adenoma or carcinoma in males exposed to 312 or 625 ppm exceeded the range in historical controls from 2-year NTP inhalation studies (Tables 9 and A4a).

At 15 months, renal tubule hyperplasia was observed in one female exposed to 625 ppm. At the end of the study, the incidence of renal tubule hyperplasia in

females in the 1,250 ppm group was significantly greater than that in the controls (Tables 9 and B5); the incidences of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) in females exposed to 1,250 ppm were also greater than those in the controls (Tables 9 and B3), and the incidences occurred with significant trends. The incidences of renal tubule adenoma or carcinoma (combined) in females exposed to 312 or 1,250 ppm exceeded the range in historical controls (Tables 9 and A4a).

Initially, a single hematoxylin- and eosin-stained section of each kidney was prepared. Primarily because of the increased incidences of renal tubule hyperplasia, adenomas, and carcinomas that were observed in some groups in the standard evaluation of the 2-year studies, additional step sections of kidney were prepared from the remaining formalin-fixed tissues of interim evaluation and 2-year study rats. Six to ten additional kidney sections taken at 1-mm intervals were prepared for each male and female. Additional males and females with focal hyperplasia or adenomas and one 156 ppm male with a carcinoma were identified among rats exposed for 2 years. The incidences of these proliferative lesions in standard and extended evaluations are presented in Table 9.

At the 2-year extended evaluations and combined standard and extended evaluations, males in the 625 ppm group and females in the 1,250 ppm group had significantly greater incidences of renal tubule hyperplasia, renal tubule adenoma, and renal tubule adenoma or carcinoma (combined) than the controls (Tables 9 and A3). In the combined standard and extended evaluations, the incidences of renal tubule hyperplasia in females exposed to 625 ppm and renal tubule adenoma in males exposed to 312 ppm were also significantly greater than those in the controls. The incidences of renal tubule adenoma in females and of renal tubule adenoma or carcinoma (combined) in males and females occurred with significant positive trends in the extended and combined standard and extended evaluations.

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|---|-----------------------|------------|------------|------------|
| Male | | | | |
| 15-Month Interim Evaluation | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 |
| Single Sections (Standard Evaluation) | | | | |
| Nephropathy ^a | 10 (1.8) ^b | 10 (1.8) | 10 (1.6) | 10 (2.4) |
| Renal Tubule, Degeneration | 1 (1.0) | 8** (1.0) | 10** (2.0) | 10** (3.0) |
| Renal Tubule, Hyperplasia | 0 | 0 | 1 (1.0) | 1 (1.0) |
| Renal Tubule, Carcinoma | 0 | 0 | 0 | 1 |
| Step Sections (Extended Evaluation) | | | | |
| Renal Tubule, Hyperplasia | 0 | 0 | 1 (1.0) | 0 |
| 2-Year Study | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Single Sections (Standard Evaluation) | | | | |
| Nephropathy | 49 (2.3) | 50 (1.9) | 50 (2.7) | 50 (3.5) |
| Renal Tubule, Degeneration | 2 (1.0) | 20** (1.1) | 50** (2.3) | 49** (3.6) |
| Renal Tubule, Hyperplasia | 1 (1.0) | 1 (1.0) | 1 (4.0) | 6* (1.3) |
| Renal Tubule, Hyperplasia, Oncocytic | 0 | 0 | 1 (1.0) | 0 |
| Renal Tubule, Adenoma | 0 | 0 | 6* | 3 |
| Renal Tubule, Carcinoma | 1 | 0 | 2 | 0 |
| Renal Tubule, Adenoma or Carcinoma ^c | 1 | 0 | 6 | 3 |
| Step Sections (Extended Evaluation) | | | | |
| Renal Tubule, Hyperplasia | 7 | 10 | 7 | 18** |
| Renal Tubule, Adenoma | 2 | 4 | 3 | 11** |
| Renal Tubule, Carcinoma | 0 | 1 | 0 | 0 |
| Renal Tubule, Adenoma or Carcinoma | 2 | 5 | 3 | 11** |
| Single and Step Sections (Combined) | | | | |
| Renal Tubule, Hyperplasia | 7 | 11 | 7 | 24** |
| Renal Tubule, Adenoma | 2 | 4 | 9* | 13** |
| Renal Tubule, Carcinoma | 1 | 1 | 2 | 0 |
| Renal Tubule, Adenoma or Carcinoma | 3 | 5 | 9 | 13** |

(continued)

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Inhalation Study of Tetrafluoroethylene (continued)

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|----------|------------|------------|
| Female | | | | |
| 15-Month Interim Evaluation | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 |
| Single Sections (Standard Evaluation) | | | | |
| Nephropathy | 8 (1.0) | 9 (1.0) | 9 (1.2) | 10 (1.0) |
| Renal Tubule, Degeneration | 0 | 0 | 10** (2.0) | 10** (2.6) |
| Renal Tubule, Hyperplasia | 0 | 0 | 1 (1.0) | 0 |
| Renal Tubule, Hyperplasia, Oncocytic | 0 | 0 | 0 | 1 (1.0) |
| Renal Tubule, Adenoma | 0 | 0 | 0 | 1 |
| Mixed Tumor, Malignant | 0 | 0 | 0 | 1 |
| Step Sections (Extended Evaluation) | | | | |
| Renal Tubule, Hyperplasia | 0 | 1 | 0 | 4* |
| Renal Tubule, Adenoma | 0 | 0 | 0 | 2 |
| 2-Year Study | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Single Sections (Standard Evaluation) | | | | |
| Nephropathy | 48 (1.7) | 46 (1.5) | 48 (1.7) | 47 (2.0) |
| Renal Tubule, Degeneration | 0 | 0 | 35** (1.3) | 46** (2.0) |
| Renal Tubule, Hyperplasia | 1 (2.0) | 3 (1.7) | 6 (1.2) | 12** (1.8) |
| Renal Tubule, Hyperplasia, Oncocytic | 0 | 0 | 0 | 3 (1.0) |
| Renal Tubule, Adenoma | 0 | 3 | 1 | 3 |
| Renal Tubule, Carcinoma | 0 | 0 | 0 | 2 |
| Renal Tubule, Adenoma or Carcinoma ^d | 0 | 3 | 1 | 5** |
| Step Sections (Extended Evaluation) | | | | |
| Renal Tubule, Hyperplasia | 2 | 3 | 7 | 17** |
| Renal Tubule, Adenoma | 0 | 0 | 2 | 5* |
| Renal Tubule, Carcinoma | 0 | 0 | 0 | 1 |
| Renal Tubule, Adenoma or Carcinoma | 0 | 0 | 2 | 5* |
| Single Sections and Step Sections (Combined) | | | | |
| Renal Tubule, Hyperplasia | 3 | 6 | 11* | 25** |
| Renal Tubule, Adenoma | 0 | 3 | 3 | 8** |
| Renal Tubule, Carcinoma | 0 | 0 | 0 | 3 |
| Renal Tubule, Adenoma or Carcinoma | 0 | 3 | 3 | 10** |

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (15-month interim evaluation) or the logistic regression test (2-year study)

** $P \leq 0.01$

^a Number of animals with lesion

^b Average severity grade of lesion in affected animals: 1=minimal; 2=mild; 3=moderate; 4=marked

^c Historical incidence for 2-year NTP inhalation studies with chamber control groups at all labs (mean \pm standard deviation): 6/652 (0.9% \pm 1.3%); range, 0%-4%. Historical incidence for 2-year NTP inhalation studies with chamber control groups at Battelle Pacific Northwest Laboratories: 5/347 (1.4% \pm 1.5%); range, 0%-4%

^d Historical incidence at all labs: 2/650 (0.3% \pm 0.8%); range, 0%-2%. Historical incidence at Battelle Pacific Northwest Laboratories: 2/346 (0.6% \pm 1.0%); range, 0%-2%

Renal tubule hyperplasia, as defined in the present study, was distinguished from regenerative epithelial changes commonly seen as a part of nephropathy and was considered a preneoplastic lesion. Renal tubule hyperplasia, adenoma, and carcinoma constitute a morphologic continuum. Hyperplasia was generally a focal, minimal to mild lesion consisting of tubules that were dilated to 1.5 to 2 times the normal diameter and were lined by increased numbers of tubule epithelial cells, which partially or totally filled the tubule lumen (Plate 1). Cells within hyperplastic lesions varied slightly in size and sometimes stained more basophilic than normal cells, but otherwise appeared similar to normal tubule epithelial cells. Renal tubule adenomas were larger, discrete lesions, ranging from greater than five tubule diameters to 1 mm or more in size (Plate 2). Cells within adenomas were mildly to moderately pleomorphic, sometimes had vacuolated cytoplasm, and tended to form complex patterns, particularly microtubular structures. Renal tubule carcinomas were differentiated from adenomas in that they usually were larger and less discrete and had a more prominent vascular supply and more anaplasia and cellular atypia (Plate 3). Renal tubule cell carcinoma was characterized by vesiculate nuclei with prominent nucleoli and increased numbers of mitotic figures (Plate 4).

Oncocytic hyperplasia was observed at the end of the study in one male exposed to 312 ppm and in three females exposed to 1,250 ppm (Tables 9, A5, and B5). This lesion was characterized by individual tubules or small clusters of tubules that were somewhat dilated and totally filled by large polygonal cells with abundant brightly eosinophilic granular cytoplasm and small, centrally located, basophilic nuclei (oncocytes). These lesions are thought to arise from the distal tubule epithelium.

At 15 months and at the end of the study, the incidences of renal tubule degeneration in all exposed groups of males and in females in the 625 and 1,250 ppm groups were greater than those in the controls (Tables 9, A5, and B5). Renal tubule degeneration was similar to that observed in the 13-week study. Degeneration was characterized by dilatation of renal tubule epithelial cells and was located predominantly at the corticomedullary junction (Plates 5 and 6). Dilated tubules sometimes contained eosinophilic debris and/or proteinaceous fluid in their lumens. The tubules were lined by flattened to

cuboidal epithelium (Plate 6) and often had both basophilic and eosinophilic cells lining adjacent areas of the same tubule. At 15 months, renal tubule degeneration was easily detected; however, in the 2-year study, it was sometimes obscured by the presence of concurrent nephropathy. The location of the renal tubule degeneration at the corticomedullary junction was the primary feature which distinguished this lesion from chronic progressive nephropathy. Also lacking in tetrafluoroethylene-induced renal tubule degeneration, but present in chronic progressive nephropathy, were thickened basement membranes, interstitial fibrosis, inflammatory infiltrates, and prominent protein casts in dilated tubules throughout the cortex and medulla.

The severity of nephropathy generally increased with increasing exposure concentration in male and female rats at 15 months and 2 years, although the increase in exposed female rats was not as pronounced. These increases were observed at the end of the 2-year study in males exposed to 312 or 625 ppm and in females exposed to 1,250 ppm.

Liver: The absolute and relative liver weights of females in the 1,250 ppm group and the absolute liver weight of females exposed to 625 ppm were significantly greater than those of the controls at the 15-month interim evaluation (Table F3). At 15 months, all exposed groups of males had significantly greater incidences of clear cell foci than the controls; females exposed to 625 or 1,250 ppm had significantly greater incidences of mixed cell foci than the controls (Tables 10, A5, and B5). At the end of the study, the incidences of eosinophilic foci in all exposed groups of males and of mixed cell foci and basophilic foci in males exposed to 312 or 625 ppm were significantly greater than those in the controls, as was the incidence of mixed cell foci in females exposed to 1,250 ppm. The incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in males exposed to 312 ppm were significantly greater than those in the controls at the end of the study (Tables 10 and A3). At 2 years, the incidences of hepatocellular adenoma and adenoma or carcinoma (combined) in all exposed groups of females were significantly greater than those in the controls, and the incidences occurred with significant positive trends (Tables 10 and B3). Additionally, females exposed to 312 or 625 ppm had significantly

greater incidences of hepatocellular carcinoma than the controls at 2 years. The combined incidence of hepatocellular neoplasms in each exposed group of males and females exceeded the historical control ranges in chamber controls from 2-year NTP inhalation studies (Tables 10, A4b, and B4b). Also at 2 years, the incidence of hemangiosarcoma in females exposed to 625 ppm was significantly greater than in the controls. The incidence of liver hemangiosarcoma in 625 ppm females exceeded the historical range for hemangiosarcomas in all organs in control female rats from NTP 2-year inhalation studies (Table 10), and liver hemangiosarcomas have not been observed in historical control females.

Hepatocellular foci are considered potential preneoplastic lesions. Hepatocellular foci were relatively

discrete aggregates of often enlarged hepatocytes, and the lobular pattern of the liver was generally retained. Hepatocellular adenomas consisted of nodules of hepatocytes which compressed adjacent hepatic parenchyma and lacked the normal lobular and sinusoidal pattern (Plate 7). Hepatocellular carcinomas consisted of solid sheets of hepatocytes or trabeculae, three or more cells thick (Plate 8). Neoplastic hepatocytes were anaplastic, with prominent nuclei containing one or more nucleoli and variable amounts of cytoplasm. Hemangiosarcomas were malignant vascular neoplasms characterized by irregular, vascular spaces filled with erythrocytes (Plate 9) and lined by variable numbers of pleomorphic endothelial

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--|------------|------------|-------------|------------|
| Male | | | | |
| 15-Month Interim Evaluation | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 |
| Basophilic Focus ^a | 6 | 4 | 7 | 4 |
| Clear Cell Focus | 4 | 10** | 9* | 10** |
| Cystic Degeneration | 0 | 1 | 3 | 0 |
| Eosinophilic Focus | 1 | 0 | 0 | 1 |
| Mixed Cell Focus | 0 | 2 | 2 | 2 |
| Hepatocellular Adenoma | 0 | 0 | 1 | 0 |
| Hepatocellular Carcinoma | 0 | 0 | 0 | 1 |
| 2-Year Study | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Basophilic Focus | 22 | 19 | 33* | 29** |
| Clear Cell Focus | 7 | 8 | 11 | 3 |
| Eosinophilic Focus | 3 | 18** | 22** | 19** |
| Cystic Degeneration | 17 | 39** | 35** | 32** |
| Mixed Cell Focus | 5 | 5 | 16** | 13** |
| Hepatocellular Adenoma | 3 | 6 | 8 | 5 |
| Hepatocellular Carcinoma | 1 | 1 | 10** | 3 |
| Hepatocellular Adenoma or Carcinoma ^b | | | | |
| Overall rate ^c | 4/50 (8%) | 7/50 (14%) | 15/50 (30%) | 8/50 (16%) |
| Adjusted rate ^d | 16.3% | 34.4% | 62.1% | 100.0% |
| Terminal rate ^e | 2/17 (12%) | 3/12 (25%) | 9/17 (53%) | 1/1 (100%) |
| First incidence (days) | 624 | 600 | 617 | 572 |
| Logistic regression test ^f | P=0.025 | P=0.223 | P=0.003 | P=0.112 |

(continued)

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Inhalation Study of Tetrafluoroethylene (continued)

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|----------------|------------|-------------|------------|
| Female | | | | |
| 15-Month Interim Evaluation | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 |
| Angiectasis | 0 | 1 | 0 | 0 |
| Basophilic Focus | 9 | 9 | 10 | 10 |
| Clear Cell Focus | 0 | 2 | 3 | 3 |
| Eosinophilic Focus | 0 | 5* | 2 | 0 |
| Mixed Cell Focus | 0 | 1 | 6** | 4* |
| 2-Year Study | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Angiectasis | 0 | 9** | 9** | 14** |
| Basophilic Focus | 41 | 38 | 41 | 37 |
| Clear Cell Focus | 10 | 3 | 12 | 9 |
| Eosinophilic Focus | 1 | 4 | 5* | 4 |
| Mixed Cell Focus | 12 | 14 | 16 | 18* |
| Hepatocellular Adenoma | 0 | 4* | 5** | 6** |
| Hepatocellular Carcinoma | 0 | 4* | 9** | 2 |
| Hepatocellular Adenoma or Carcinoma ^g | | | | |
| Overall rate | 0/50 (0%) | 7/50 (14%) | 12/50 (24%) | 8/50 (16%) |
| Adjusted rate | 0.0% | 36.3% | 65.4% | 37.8% |
| Terminal rate | 0/28 (0%) | 5/16 (31%) | 9/15 (60%) | 5/18 (28%) |
| First incidence (days) | — ^h | 583 | 668 | 677 |
| Logistic regression test | P=0.001 | P=0.002 | P<0.001 | P<0.001 |
| Hemangiosarcoma ⁱ | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 5/50 (10%) | 1/50 (2%) |
| Adjusted rate | 0.0% | 0.0% | 19.5% | 5.0% |
| Terminal rate | 0/28 (0%) | 0/16 (0%) | 0/15 (0%) | 0/18 (0%) |
| First incidence (days) | — | — | 589 | 703 |
| Logistic regression test | P=0.182 | — | P=0.025 | P=0.435 |

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (15-month interim evaluation) or the logistic regression test (2-year study)

** $P \leq 0.01$

^a Number of animals with lesion

^b Historical incidence for 2-year NTP inhalation studies with chamber control groups at all labs (mean \pm standard deviation): 28/653 (4.3% \pm 2.9%); range, 2%-9%. Historical incidence for 2-year NTP inhalation studies with chamber control groups at Battelle Pacific Northwest Laboratories: 11/347 (3.2% \pm 2.3%); range, 2%-8%

^c Number of animals with neoplasm per number of animals with liver examined microscopically

^d Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

^e Observed incidence in animals surviving until the end of the study

^f In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal.

^g Historical incidence at all labs: 10/650 (1.5% \pm 2.0%); range, 0%-6%. Historical incidence at Battelle Pacific Northwest Laboratories: 7/346 (2.0% \pm 2.3%); range, 0%-6%

^h Not applicable; no neoplasms in animal group

ⁱ Historical incidence for all organs at all labs: 2/653 (0.3% \pm 0.8%); range, 0%-2% (incidence in liver: 0/650)

cells. Occasionally, hemangiosarcomas metastasized to the lung.

At the end of the 2-year study, increased incidences of hepatic cystic degeneration occurred in all exposed groups of males, and increased incidences of hepatic angiectasis were observed in exposed groups of females (Tables 10, A5, and B5). Cystic degeneration was characterized by multifocal dilated areas with cysts containing fluid, fibrin, and/or erythrocytes; these lesions occur spontaneously in aging rats at low incidences but are common following chemical exposure to a hepatocarcinogen. Hepatic angiectasis was characterized by multifocal, dilated, blood-filled sinusoids with prominent lining endothelial cells; these lesions are sometimes associated with hepatocellular neoplasms.

Mononuclear Cell Leukemia: Incidences of mononuclear cell leukemia in males exposed to 156 ppm and in all exposed groups of females were significantly greater than those in the controls (Tables 11, A3, and B3), and the incidences occurred with significant positive trends in males and females. The incidence of this neoplasm in male controls exceeded the historical range for all types of leukemia in 2-year NTP inhalation studies (Tables 11 and A4c), as did the incidences in males exposed to 156 or 312 ppm. However, the incidence in females exposed to 0 ppm was within the historical control range (Tables 11 and B4c), and the incidences in females exposed to 312 or 1,250 ppm exceeded that range.

Eye: The incidence of cataracts in females exposed to 1,250 ppm was greater than that in the controls at the

end of the 2-year study (0 ppm, 15/50; 312 ppm, 4/50; 625 ppm, 10/50; 1,250 ppm, 45/50; Table B5). Cataracts were characterized by a disruption of the normal organization of lens fibers, with swelling, vacuolization, and mineralization.

Testis: At the end of the study, there was a slight increase in the incidences of interstitial cell adenoma in rats exposed to 312 or 625 ppm (39/50, 40/50, 48/50, 47/50; Table A3). At 15 months, there were no increases in the incidences of interstitial cell hyperplasia or adenoma. Interstitial cell hyperplasia and adenomas are very common in F344/N rats, and nearly all rats will develop such proliferative lesions if allowed to complete their natural life span. It is uncertain whether the slightly increased incidences of interstitial cell adenoma were related to tetrafluoroethylene exposure, although the control incidence is within the historical control range for 2-year NTP inhalation studies with chamber controls (450/655; mean \pm standard deviation, 68.7% \pm 8.7%; range, 54%-83%).

Mammary Gland: At 2 years, the incidences of fibroadenoma in the mammary gland of exposed female rats were decreased (22/50, 11/50, 9/50, 7/50; Table B3). Fibroadenomas are the most common benign neoplasm of the mammary gland in the female F344/N rat. In other studies, decreases in the incidence of this neoplasm have been associated with decreases in body weights; however, in the present study, the decreases are not related to body weight changes. The biological significance of these decreased incidences is uncertain.

TABLE 11
Incidences of Mononuclear Cell Leukemia in Rats in the 2-Year Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|---|-------------|-------------|-------------|-------------|
| Male | | | | |
| Mononuclear Cell Leukemia (All Organs) ^a | | | | |
| Overall rate ^b | 34/50 (68%) | 43/50 (86%) | 38/50 (76%) | 31/50 (62%) |
| Adjusted rate ^c | 81.5% | 95.3% | 91.9% | 100.0% |
| Terminal rate ^d | 10/17 (59%) | 10/12 (83%) | 14/17 (82%) | 1/1 (100%) |
| First incidence (days) | 509 | 351 | 413 | 509 |
| Life table test ^e | P<0.001 | P=0.016 | P=0.231 | P<0.001 |
| Logistic regression test ^e | P=0.064N | P=0.020 | P=0.254 | P=0.079N |
| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
| Female | | | | |
| Mononuclear Cell Leukemia (All Organs) ^f | | | | |
| Overall rate | 16/50 (32%) | 31/50 (62%) | 23/50 (46%) | 36/50 (72%) |
| Adjusted rate | 43.7% | 76.5% | 65.1% | 82.6% |
| Terminal rate | 8/28 (29%) | 7/16 (44%) | 6/15 (40%) | 11/18 (61%) |
| First incidence (days) | 621 | 468 | 469 | 372 |
| Life table test | P<0.001 | P<0.001 | P=0.008 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P=0.105 | P<0.001 |

^a Historical incidence for 2-year NTP inhalation studies with chamber control groups at all labs (mean ± standard deviation): 356/655 (54.4% ± 8.8%); range, 34%-66%. Historical incidence for 2-year NTP inhalation studies with chamber control groups at Battelle Pacific Northwest Laboratories: 195/348 (56.0% ± 8.7%); range, 38%-66%

^b Number of animals with neoplasm per number of animals necropsied

^c Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

^d Observed incidence in animals surviving until the end of the study

^e In the control column are the P values associated with the trend test. In the exposed group columns 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 neoplasms as nonfatal. A negative trend or a lower incidence in an exposure group is indicated by N.

^f Historical incidence at all labs: 262/653 (40.1% ± 7.2%); range, 30%-54%. Historical incidence at Battelle Pacific Northwest Laboratories: 146/348 (42.0% ± 7.2%); range, 30%-54%

MICE

16-DAY STUDY

All mice survived to the end of the study (Table 12). Final mean body weights and body weight gains of all exposed groups of mice were similar to those of the controls. There were no exposure-related clinical findings in male or female mice.

There were no significant differences in hematology parameters that were considered to be related to tetrafluoroethylene exposure (Table G4). The absolute and relative liver weights of females exposed to 5,000 ppm were significantly greater than those of the controls, as were the absolute kidney weight of females in that group and the absolute liver weight of females in the 2,500 ppm group (Table F4). Other differences in organ weights were not considered to be related to chemical exposure.

Renal tubule karyomegaly was observed in male mice exposed to 1,250 or greater, in females in the 2,500 and 5,000 ppm groups, and in one female exposed to 1,250 ppm; the severity of this lesion increased with increasing exposure concentration (Table 13). Karyomegaly of renal tubule cells was observed primarily in the inner renal cortex and was characterized by nuclei that were enlarged, pleomorphic, and vesicular and that had prominent nucleoli. In the areas with karyomegaly, individual tubule epithelial cells had pyknotic nuclei consistent with minimal to mild scattered necrosis.

Exposure Concentration Selection Rationale: Based on the lack of overt toxicity following exposure of mice to 5,000 ppm for 16 days, exposure concentrations selected for the 13-week study were 0, 312, 625, 1,250, 2,500, and 5,000 ppm.

TABLE 12
Survival and Body Weights of Mice in the 16-Day Inhalation Study of Tetrafluoroethylene

| Exposure Concentration (ppm) | Survival ^a | Mean Body Weight ^b (g) | | | Final Weight Relative to Controls (%) |
|------------------------------|-----------------------|-----------------------------------|------------|-----------|---------------------------------------|
| | | Initial | Final | Change | |
| Male | | | | | |
| 0 | 5/5 | 22.4 ± 0.3 | 25.3 ± 0.2 | 3.0 ± 0.3 | |
| 312 | 5/5 | 22.6 ± 0.7 | 25.5 ± 0.7 | 2.9 ± 0.3 | 101 |
| 625 | 5/5 | 22.2 ± 0.5 | 24.8 ± 0.6 | 2.6 ± 0.4 | 98 |
| 1,250 | 5/5 | 21.5 ± 0.7 | 24.8 ± 1.2 | 3.4 ± 0.6 | 98 |
| 2,500 | 5/5 | 21.8 ± 0.7 | 25.7 ± 0.5 | 3.9 ± 0.2 | 102 |
| 5,000 | 5/5 | 21.8 ± 1.0 | 25.2 ± 1.0 | 3.4 ± 0.3 | 100 |
| Female | | | | | |
| 0 | 5/5 | 18.8 ± 0.7 | 20.8 ± 0.6 | 2.0 ± 0.6 | |
| 312 | 5/5 | 18.8 ± 1.2 | 20.8 ± 1.1 | 2.0 ± 0.3 | 100 |
| 625 | 5/5 | 18.8 ± 0.3 | 20.9 ± 0.3 | 2.1 ± 0.1 | 101 |
| 1,250 | 5/5 | 18.2 ± 1.0 | 21.0 ± 0.8 | 2.7 ± 0.5 | 101 |
| 2,500 | 5/5 | 18.9 ± 0.6 | 21.5 ± 0.7 | 2.6 ± 0.5 | 104 |
| 5,000 | 5/5 | 18.2 ± 0.4 | 21.3 ± 0.6 | 3.1 ± 0.5 | 103 |

^a Number of animals surviving at 16 days/number initially in group

^b Weights and weight changes are given as mean ± standard error. Differences from the control group were not significant by Williams' or Dunnett's test.

TABLE 13
Incidences of Kidney Lesions in Mice in the 16-Day Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|-------|---------|---------|------------------------|-----------|-----------|
| Male | | | | | | |
| Number Examined Microscopically | 5 | 5 | 5 | 5 | 5 | 5 |
| Renal Tubule, Karyomegaly ^a | 0 | 0 | 0 | 5** (1.0) ^b | 5** (1.2) | 5** (2.4) |
| Female | | | | | | |
| Number Examined Microscopically | 5 | 5 | 5 | 5 | 5 | 5 |
| Renal Tubule, Karyomegaly | 0 | 0 | 0 | 1 (1.0) | 5** (2.0) | 5** (3.0) |

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test

^a Number of animals with lesion

^b Average severity grade of lesions in affected mice: 1=minimal; 2=mild; 3=moderate; 4=marked

13-WEEK STUDY

All mice survived to the end of the study (Table 14). Males in the 1,250, 2,500, and 5,000 ppm groups had significantly lower initial mean body weights than the controls; however, final mean body weights and body weight gains of all exposed groups of male and female mice were generally similar to those of the controls. There were no clinical findings that were considered related to tetrafluoroethylene exposure.

The hematology and urinalysis data for mice in the 13-week inhalation study of tetrafluoroethylene are presented in Table G5. A normocytic, normochromic, nonresponsive anemia, similar to that observed in the 13-week rat study, occurred in males exposed to 2,500 ppm and in males and females in the 5,000 ppm groups. The anemia was evidenced by minimal decreases of hematocrit values, hemoglobin concentrations, and erythrocyte counts. There were

no alterations of mean cell volume or mean cell hemoglobin concentration; thus, the erythrocytes were normocytic and normochromic. The lack of a bone marrow response was evidenced by the absence of an increase in reticulocyte counts.

A concentration-dependent polyuria, evidenced by increased 16-hour urine volumes, occurred in male and female mice exposed to 2,500 or 5,000 ppm. The increased urine volumes were accompanied by decreased urine specific gravity values; these findings are consistent with increased water consumption or lack of urine concentrating ability of the kidney. There was a concentration-dependent increase of urinary fluoride excretion in all exposed groups of male and female mice, consistent with metabolism and excretion of tetrafluoroethylene. No biologically significant differences in organ weights were observed.

TABLE 14
Survival and Body Weights of Mice in the 13-Week Inhalation Study of Tetrafluoroethylene

| Exposure Concentration (ppm) | Survival ^a | Mean Body Weight ^b (g) | | | Final Weight Relative to Controls (%) |
|------------------------------|-----------------------|-----------------------------------|-------------|-----------|---------------------------------------|
| | | Initial | Final | Change | |
| Male | | | | | |
| 0 | 10/10 | 25.5 ± 0.3 | 33.6 ± 0.6 | 8.2 ± 0.5 | |
| 312 | 10/10 | 24.9 ± 0.3 | 32.4 ± 0.3 | 7.4 ± 0.3 | 96 |
| 625 | 10/10 | 25.0 ± 0.4 | 33.6 ± 0.7 | 8.6 ± 0.5 | 100 |
| 1,250 | 10/10 | 24.0 ± 0.2** | 33.3 ± 0.6 | 9.3 ± 0.4 | 99 |
| 2,500 | 10/10 | 24.8 ± 0.2** | 32.6 ± 0.3 | 7.9 ± 0.4 | 97 |
| 5,000 | 10/10 | 24.0 ± 0.2** | 31.6 ± 0.5* | 7.6 ± 0.4 | 94 |
| Female | | | | | |
| 0 | 10/10 | 20.2 ± 0.3 | 28.8 ± 0.8 | 8.7 ± 0.7 | |
| 312 | 10/10 | 20.0 ± 0.2 | 28.5 ± 0.7 | 8.4 ± 0.6 | 99 |
| 625 | 10/10 | 20.1 ± 0.4 | 27.8 ± 0.9 | 7.7 ± 0.8 | 96 |
| 1,250 | 10/10 | 19.7 ± 0.1 | 27.5 ± 0.6 | 7.8 ± 0.6 | 95 |
| 2,500 | 10/10 | 19.9 ± 0.2 | 27.8 ± 0.7 | 7.9 ± 0.6 | 96 |
| 5,000 | 10/10 | 19.9 ± 0.1 | 26.3 ± 0.4 | 6.4 ± 0.4 | 91 |

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

** $P \leq 0.01$

^a Number of animals surviving at 13 weeks/number initially in group

^b Weights and weight changes are given as mean ± standard error.

Differences in sperm morphology parameters and estrous cycle length were not considered to be related to chemical administration (Tables H3 and H4).

Incidences of karyomegaly of the renal tubule epithelial cells in male and female mice exposed to 1,250 ppm or greater were significantly greater than those in the controls (Table 15). As in the 16-day study, the renal changes were observed primarily in the inner renal cortex. Karyomegaly was characterized by nuclei that were enlarged, pleomorphic, and vesicular and had large, hyperchromatic, prominent nucleoli. In areas of karyomegaly, there was minimal cytomegaly and scattered necrosis of individual

tubule epithelial cells. The severity of the kidney lesions was slightly more pronounced in males than in females.

Exposure Concentration Selection Rationale: Based on the incidence and severity of kidney lesions in male and female mice exposed to 2,500 or 5,000 ppm tetrafluoroethylene, the absence of kidney lesions in mice exposed to 625 ppm, and the absence of kidney weight effects in exposed groups of mice, exposure concentrations selected for the 2-year mouse study were 312, 625, and 1,250 ppm. The highest concentration (1,250 ppm) was expected to cause minimal toxicity in the 2-year study.

TABLE 15
Incidences of Nonneoplastic Lesions of the Kidney in Mice in the 13-Week Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---------------------------------|-------|---------|---------|-------------------------|------------|------------|
| Male | | | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 | 10 | 10 |
| Karyomegaly ^a | 0 | 0 | 0 | 10** (2.8) ^b | 10** (2.8) | 10** (3.0) |
| Female | | | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 | 10 | 10 |
| Karyomegaly | 0 | 0 | 0 | 10** (1.8) | 10** (2.8) | 10** (2.2) |

** Significantly different ($P < 0.01$) from the control group by the Fisher exact test

^a Number of animals with lesion

^b Average severity grade of lesions in affected mice: 1=minimal; 2=mild; 3=moderate; 4=marked

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 16 and in the Kaplan-Meier survival curves (Figure 3). The survival rates of all exposed groups of males and females were significantly less than those of the controls. Because of the reduced survival, the study was terminated during week 96.

Body Weights and Clinical Findings

Mean body weights of exposed groups were generally similar to those of the controls except at the end of the study, when they were somewhat lower; however, the number of exposed mice surviving at the end of the study was considerably less than the number of controls surviving (Tables 17 and 18 and Figure 4). There were no clinical findings related to tetrafluoroethylene exposure.

TABLE 16
Survival of Mice in the 2-Year Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|---------|---------|---------|-----------|
| Male | | | | |
| Animals initially in study | 58 | 58 | 58 | 58 |
| 15-Month interim evaluation ^a | 10 | 10 | 10 | 10 |
| Moribund | 6 | 24 | 26 | 27 |
| Natural deaths | 4 | 13 | 20 | 20 |
| Animals surviving to study termination | 38 | 11 | 2 | 1 |
| Percent probability of survival at end of study ^b | 79 | 23 | 4 | 2 |
| Mean survival (days) ^c | 647 | 561 | 554 | 548 |
| Survival analyses ^d | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Female | | | | |
| Animals initially in study | 58 | 58 | 58 | 58 |
| 15-Month interim evaluation ^a | 10 | 10 | 10 | 10 |
| Accidental death ^a | 0 | 0 | 1 | 0 |
| Moribund | 8 | 28 | 26 | 27 |
| Natural deaths | 4 | 16 | 15 | 17 |
| Animals surviving to study termination | 36 | 4 | 6 | 4 |
| Percent probability of survival at end of study | 75 | 8 | 13 | 8 |
| Mean survival (days) | 643 | 597 | 587 | 560 |
| Survival analyses | P<0.001 | P<0.001 | P<0.001 | P<0.001 |

^a Censored from survival analyses

^b Kaplan-Meier determinations

^c Mean of all deaths (uncensored, censored, and terminal sacrifice)

^d The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns.

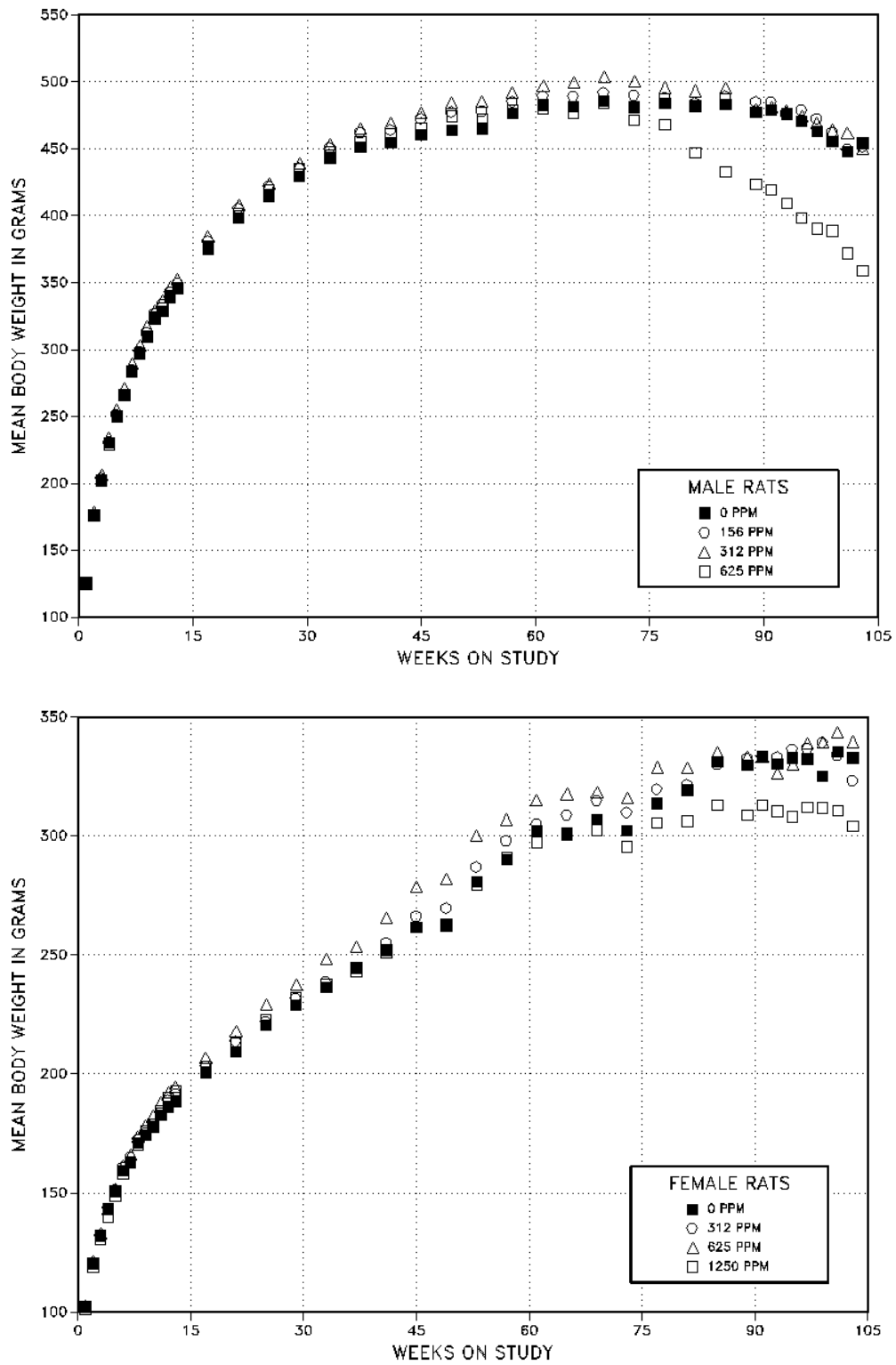


FIGURE 2
Growth Curves for Male and Female Rats Administered Tetrafluoroethylene by Inhalation for 2 Years

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

| Weeks on Study | 0 ppm | | 312 ppm | | | 625 ppm | | | 1,250 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 | 24.1 | 58 | 24.0 | 100 | 58 | 24.0 | 100 | 58 | 23.8 | 99 | 58 |
| 2 | 26.6 | 58 | 26.7 | 100 | 58 | 26.9 | 101 | 58 | 26.9 | 101 | 57 |
| 3 | 27.4 | 58 | 27.7 | 101 | 58 | 27.7 | 101 | 58 | 27.8 | 102 | 57 |
| 4 | 27.7 | 58 | 28.2 | 102 | 58 | 28.5 | 103 | 58 | 28.3 | 102 | 57 |
| 5 | 29.0 | 58 | 29.8 | 103 | 58 | 29.8 | 103 | 58 | 29.9 | 103 | 57 |
| 6 | 29.7 | 58 | 30.5 | 103 | 58 | 30.7 | 103 | 58 | 30.7 | 103 | 57 |
| 7 | 30.2 | 58 | 30.8 | 102 | 58 | 31.3 | 104 | 58 | 31.6 | 105 | 57 |
| 8 | 30.9 | 58 | 31.9 | 103 | 57 | 31.9 | 103 | 58 | 32.2 | 104 | 57 |
| 9 | 31.8 | 58 | 32.6 | 103 | 57 | 33.0 | 104 | 58 | 32.9 | 104 | 57 |
| 10 | 32.3 | 58 | 33.1 | 103 | 57 | 33.6 | 104 | 58 | 33.4 | 103 | 57 |
| 11 | 32.9 | 58 | 33.7 | 102 | 57 | 34.3 | 104 | 58 | 34.2 | 104 | 57 |
| 12 | 33.6 | 58 | 34.5 | 103 | 55 | 34.6 | 103 | 58 | 34.6 | 103 | 57 |
| 13 | 33.4 | 58 | 34.2 | 102 | 55 | 34.1 | 102 | 58 | 34.7 | 104 | 57 |
| 14 | 35.2 | 58 | 35.9 | 102 | 55 | 35.8 | 102 | 58 | 36.2 | 103 | 57 |
| 18 | 37.0 | 58 | 37.9 | 102 | 55 | 38.4 | 104 | 58 | 38.1 | 103 | 57 |
| 22 | 39.1 | 58 | 39.3 | 101 | 55 | 39.7 | 102 | 58 | 39.7 | 102 | 57 |
| 26 | 41.3 | 58 | 41.9 | 102 | 55 | 42.3 | 102 | 58 | 41.5 | 101 | 57 |
| 30 | 43.6 | 58 | 44.0 | 101 | 55 | 44.5 | 102 | 58 | 43.4 | 100 | 57 |
| 34 | 45.3 | 58 | 45.9 | 101 | 55 | 45.8 | 101 | 58 | 44.7 | 99 | 57 |
| 38 | 46.3 | 58 | 46.9 | 101 | 55 | 46.8 | 101 | 58 | 45.5 | 98 | 56 |
| 42 | 46.2 | 58 | 46.6 | 101 | 55 | 46.6 | 101 | 58 | 45.7 | 99 | 56 |
| 46 | 48.1 | 58 | 48.1 | 100 | 55 | 47.3 | 98 | 58 | 47.1 | 98 | 55 |
| 50 | 48.5 | 58 | 49.0 | 101 | 55 | 48.2 | 99 | 58 | 48.2 | 99 | 55 |
| 54 | 48.7 | 58 | 48.5 | 100 | 55 | 47.9 | 98 | 56 | 48.2 | 99 | 54 |
| 58 | 48.4 | 58 | 48.9 | 101 | 55 | 48.3 | 100 | 56 | 49.1 | 101 | 53 |
| 62 | 49.5 | 58 | 49.3 | 100 | 55 | 48.5 | 98 | 54 | 49.4 | 100 | 53 |
| 66 ^a | 49.0 | 47 | 48.1 | 98 | 45 | 47.6 | 97 | 41 | 49.3 | 101 | 43 |
| 70 | 50.2 | 46 | 49.1 | 98 | 43 | 47.8 | 95 | 40 | 49.5 | 99 | 41 |
| 74 | 50.5 | 46 | 49.3 | 98 | 40 | 47.9 | 95 | 37 | 49.0 | 97 | 41 |
| 78 | 50.1 | 45 | 49.7 | 99 | 33 | 48.8 | 97 | 28 | 48.8 | 97 | 34 |
| 82 | 50.8 | 44 | 49.5 | 97 | 29 | 49.6 | 98 | 22 | 46.8 | 92 | 26 |
| 86 | 49.8 | 42 | 48.0 | 96 | 23 | 48.3 | 97 | 16 | 46.1 | 93 | 18 |
| 90 | 49.9 | 41 | 45.9 | 92 | 17 | 47.9 | 96 | 9 | 44.6 | 89 | 7 |
| 92 | 49.6 | 40 | 44.9 | 91 | 15 | 46.5 | 94 | 6 | | | |
| 94 | 49.2 | 38 | 44.7 | 91 | 12 | | | | | | |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 30.0 | | 30.6 | 102 | | 30.8 | 103 | | 30.8 | 103 | |
| 14-52 | 43.1 | | 43.6 | 101 | | 43.5 | 101 | | 43.0 | 100 | |
| 53-94 | 49.6 | | 48.0 | 97 | | 48.1 | 97 | | 48.1 | 97 | |

^a Interim evaluation occurred during week 66.

TABLE 18
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene

| Weeks on Study | 0 ppm | | 312 ppm | | | 625 ppm | | | 1,250 ppm | | |
|-----------------------|----------------|---------------------|----------------|------------------------|---------------------|----------------|------------------------|---------------------|----------------|------------------------|---------------------|
| | Av. Wt. (g) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors |
| 1 | 19.3 | 58 | 19.2 | 100 | 58 | 19.1 | 99 | 58 | 19.2 | 100 | 58 |
| 2 | 21.6 | 58 | 21.7 | 101 | 58 | 21.6 | 100 | 58 | 21.6 | 100 | 58 |
| 3 | 22.2 | 58 | 22.3 | 101 | 58 | 22.2 | 100 | 58 | 21.8 | 98 | 58 |
| 4 | 22.6 | 58 | 22.4 | 99 | 58 | 22.5 | 100 | 58 | 22.6 | 100 | 58 |
| 5 | 23.9 | 58 | 24.0 | 100 | 58 | 23.9 | 100 | 58 | 24.1 | 101 | 58 |
| 6 | 24.4 | 58 | 24.8 | 102 | 58 | 24.6 | 101 | 58 | 24.8 | 102 | 58 |
| 7 | 25.1 | 58 | 25.0 | 100 | 58 | 25.3 | 101 | 58 | 25.1 | 100 | 58 |
| 8 | 25.3 | 58 | 25.7 | 102 | 58 | 25.6 | 101 | 58 | 25.4 | 100 | 58 |
| 9 | 26.2 | 58 | 26.2 | 100 | 58 | 26.4 | 101 | 58 | 26.3 | 100 | 58 |
| 10 | 26.8 | 58 | 26.9 | 100 | 58 | 26.9 | 100 | 58 | 26.8 | 100 | 58 |
| 11 | 27.1 | 58 | 27.3 | 101 | 58 | 27.5 | 102 | 58 | 27.3 | 101 | 58 |
| 12 | 27.3 | 58 | 28.1 | 103 | 58 | 27.5 | 101 | 58 | 27.6 | 101 | 58 |
| 13 | 27.8 | 58 | 27.9 | 100 | 58 | 27.5 | 99 | 58 | 27.7 | 100 | 58 |
| 14 | 29.2 | 58 | 29.4 | 101 | 58 | 29.6 | 101 | 58 | 29.1 | 100 | 58 |
| 18 | 30.7 | 58 | 30.9 | 101 | 58 | 32.2 | 105 | 58 | 30.5 | 99 | 58 |
| 22 | 34.3 | 58 | 34.0 | 99 | 58 | 34.6 | 101 | 58 | 33.3 | 97 | 56 |
| 26 | 36.2 | 58 | 36.6 | 101 | 58 | 37.1 | 103 | 58 | 34.4 | 95 | 56 |
| 30 | 38.0 | 58 | 39.0 | 103 | 58 | 39.7 | 105 | 58 | 37.0 | 97 | 56 |
| 34 | 40.2 | 58 | 40.5 | 101 | 58 | 41.9 | 104 | 58 | 38.3 | 95 | 56 |
| 38 | 41.7 | 58 | 42.6 | 102 | 58 | 43.7 | 105 | 58 | 39.8 | 95 | 56 |
| 42 | 43.1 | 58 | 43.4 | 101 | 58 | 44.1 | 102 | 58 | 41.8 | 97 | 56 |
| 46 | 45.0 | 58 | 45.2 | 100 | 58 | 46.0 | 102 | 58 | 44.0 | 98 | 56 |
| 50 | 45.7 | 58 | 46.0 | 101 | 58 | 47.2 | 103 | 58 | 44.2 | 97 | 56 |
| 54 | 47.1 | 58 | 47.7 | 101 | 58 | 48.7 | 103 | 58 | 45.8 | 97 | 55 |
| 58 | 48.0 | 57 | 49.6 | 103 | 58 | 49.8 | 104 | 58 | 47.3 | 99 | 55 |
| 62 | 51.0 | 57 | 51.3 | 101 | 58 | 52.1 | 102 | 58 | 49.5 | 97 | 55 |
| 66 ^a | 50.9 | 47 | 50.6 | 99 | 44 | 51.1 | 100 | 48 | 48.8 | 96 | 45 |
| 70 | 51.8 | 47 | 50.7 | 98 | 44 | 50.9 | 98 | 43 | 49.1 | 95 | 42 |
| 74 | 51.6 | 46 | 50.4 | 98 | 43 | 49.8 | 97 | 40 | 48.7 | 94 | 39 |
| 78 | 51.9 | 45 | 51.8 | 100 | 38 | 51.6 | 99 | 35 | 49.5 | 95 | 35 |
| 82 | 52.0 | 44 | 50.7 | 98 | 35 | 49.4 | 95 | 31 | 48.3 | 93 | 28 |
| 86 | 50.7 | 41 | 48.8 | 96 | 32 | 50.0 | 99 | 22 | 47.1 | 93 | 17 |
| 90 | 49.6 | 38 | 45.7 | 92 | 20 | 48.2 | 97 | 18 | 41.4 | 84 | 10 |
| 92 | 50.9 | 37 | 43.8 | 86 | 14 | 49.2 | 97 | 13 | 41.3 | 81 | 7 |
| 94 | 50.5 | 37 | | | | 50.8 | 101 | 7 | 40.1 | 79 | 4 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 24.6 | | 24.7 | 100 | | 24.7 | 100 | | 24.6 | 100 | |
| 14-52 | 38.4 | | 38.8 | 101 | | 39.6 | 103 | | 37.2 | 97 | |
| 53-94 | 50.5 | | 49.2 | 97 | | 50.1 | 99 | | 46.4 | 92 | |

^a Interim evaluation occurred during week 66.

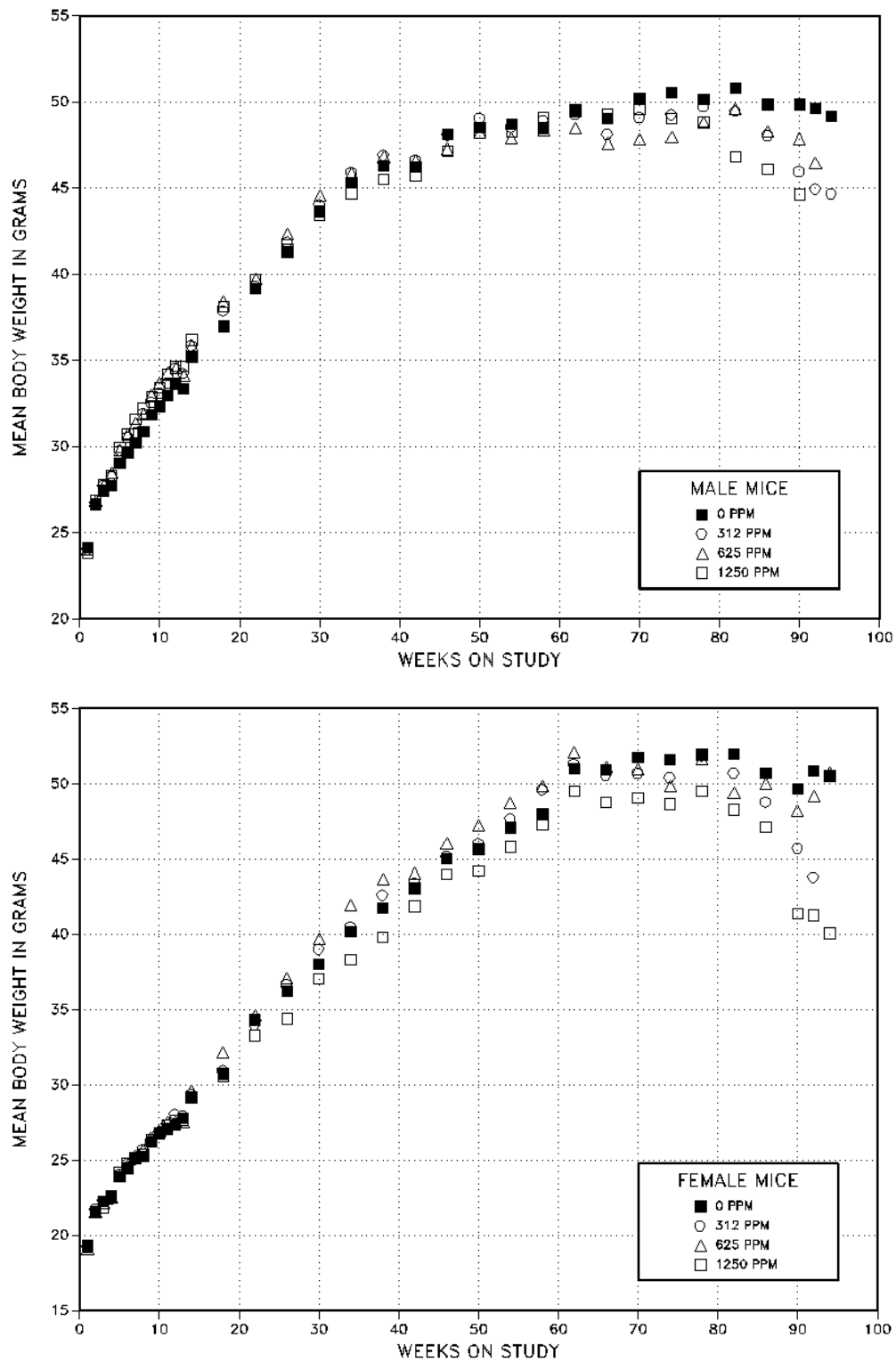


FIGURE 4
Growth Curves for Male and Female Mice Administered Tetrafluoroethylene by Inhalation for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of histiocytic sarcoma and of neoplasms and/or nonneoplastic lesions of the liver, kidney, and spleen. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasm as mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

At the 15-month interim evaluation, absolute and relative lung weights of exposed groups of mice were similar to those of the controls (Table F6), and no lung lesions related to tetrafluoroethylene exposure occurred.

Liver: At the 15-month interim evaluation, absolute and relative liver weights of exposed groups of mice were similar to those of the controls (Table F6). At 15 months and at the end of the study, vascular neoplasms and nonneoplastic lesions occurred in the livers of exposed male and female mice. At the 15-month interim evaluation, hemangiosarcomas occurred in three males exposed to 1,250 ppm and in one female exposed to 312 ppm (Tables 19, C1, and D1). At the end of the study, the incidences of hemangioma in males and females exposed to 312 ppm and in males exposed to 625 ppm were significantly greater than those in the controls (Tables 19, C3, and D3); the incidences of hemangioma in all exposed groups of males and in females exposed to 312 or 625 ppm exceeded the range in historical chamber controls from 2-year NTP inhalation studies (Tables 19, C4a, and D4a). The incidences of hemangiosarcoma in all exposed groups of males and females were significantly greater than those in the controls and exceeded the historical control ranges. The incidences occurred with a significant positive trend in males and females. Multiple hemangiomas occurred in all exposed groups of males and females except females in the 1,250 ppm group, and multiple hemangiosarcomas occurred in all exposed groups of males and females.

Hemangiomas are benign, vascular neoplasms characterized by dilated vascular spaces (Plate 10), with an increase in the number of neoplastic endothelial cells (Plate 11). Hemangiosarcomas are malignant vascular neoplasms characterized by large, irregular, cavernous spaces filled with erythrocytes and masses of fibrin (Plate 12) and lined by variable numbers of pleomorphic endothelial cells which form fronds, solid sheets (Plate 13), or vascular channels around and within extensive areas of thrombosis and necrosis. Although there were high incidences of hepatic hemangiosarcomas in all groups of exposed mice, incidences of this neoplasm in other organs were low. Hemangiosarcomas were observed in the lung, mesentery, pancreas, ovary, bone marrow, and subcutaneous tissues of a few mice exposed to tetrafluoroethylene, and all of these mice had hepatic hemangiosarcomas. It was not clear whether the few extra-hepatic hemangiosarcomas were metastases or whether they arose concomitantly in extra-hepatic sites. There were no hemangiosarcomas in the liver or in other organs of control mice.

At 15 months and at the end of the studies, increased incidences of angiectasis occurred in all exposed groups of males and females (Tables 19, C5, and D5). Angiectasis is characterized by dilated vascular spaces of sinusoids in which the endothelial cells are normal in morphology and number, and the lesion is commonly observed following exposure to a hepatocarcinogen.

At 15 months, hepatocellular adenomas and carcinomas occurred in all control and exposed groups of males, but only in exposed groups of females (Tables 19, C1, and D1). Additionally, the incidences of eosinophilic foci were significantly greater in females exposed to 625 or 1,250 ppm than in the controls (Tables 19 and D5). At the end of the study, the incidence of hepatocellular adenoma in females exposed to 625 ppm was significantly greater than that in the controls and exceeded the historical control range from 2-year NTP inhalation studies. Multiple hepatocellular adenomas occurred in all control and exposed groups of males and females, and the incidences in exposed females were significantly

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-------|---------|---------|-----------|
| Male | | | | |
| 15-Month Interim Evaluation | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 |
| Angiectasis ^a | 0 | 1 | 5* | 2 |
| Eosinophilic Focus | 0 | 0 | 3 | 0 |
| Necrosis, Coagulative, Multifocal | 1 | 1 | 0 | 1 |
| Hemangiosarcoma | 0 | 0 | 0 | 3 |
| Hepatocellular Adenoma | 6 | 2 | 4 | 1 |
| Hepatocellular Adenoma, Multiple | 1 | 0 | 2 | 0 |
| Hepatocellular Carcinoma | 2 | 4 | 2 | 2 |
| 2-Year Study | | | | |
| Number Examined Microscopically | 48 | 48 | 48 | 48 |
| Angiectasis | 0 | 6** | 10** | 13** |
| Eosinophilic Focus | 1 | 6 | 7* | 7* |
| Necrosis, Coagulative, Multifocal | 4 | 3 | 13* | 11* |
| Hemangioma ^b | 0 | 10** | 5* | 2 |
| Hemangioma, Multiple | 0 | 7** | 2 | 1 |
| Hemangiosarcoma ^c | 0 | 21** | 27** | 37** |
| Hemangiosarcoma, Multiple | 0 | 16** | 17** | 18** |
| Hemangioma or Hemangiosarcoma | 0 | 26** | 30** | 38** |
| Hepatocellular Adenoma | 17 | 17 | 12 | 20 |
| Hepatocellular Adenoma, Multiple | 6 | 6 | 7 | 7 |
| Hepatocellular Carcinoma | 11 | 20** | 33** | 26** |
| Hepatocellular Carcinoma, Multiple | 4 | 9** | 9** | 6* |
| Hepatocellular Adenoma or Carcinoma ^d | 26 | 34** | 39** | 35** |

(continued)

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Inhalation Study of Tetrafluoroethylene (continued)

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-------|---------|---------|-----------|
| Female | | | | |
| 15-Month Interim Evaluation | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 |
| Angiectasis | 0 | 4* | 2 | 1 |
| Eosinophilic Focus | 0 | 1 | 4* | 5* |
| Hemangiosarcoma | 0 | 1 | 0 | 0 |
| Hepatocellular Adenoma | 0 | 2 | 3 | 2 |
| Hepatocellular Adenoma, Multiple | 0 | 0 | 0 | 1 |
| Hepatocellular Carcinoma | 0 | 3 | 1 | 3 |
| 2-Year Study | | | | |
| Number Examined Microscopically | 48 | 48 | 47 | 47 |
| Angiectasis | 1 | 9* | 6 | 4 |
| Eosinophilic Focus | 5 | 13* | 12** | 7 |
| Hematopoietic Cell Proliferation | 3 | 19** | 13 | 15** |
| Hemangioma ^e | 0 | 5* | 2 | 1 |
| Hemangioma, Multiple | 0 | 1 | 1 | 0 |
| Hemangiosarcoma ^f | 0 | 27** | 27** | 34** |
| Hemangiosarcoma, Multiple | 0 | 8** | 12** | 15** |
| Hemangioma or Hemangiosarcoma | 0 | 31** | 28** | 35** |
| Hepatocellular Adenoma | 15 | 17 | 20* | 15 |
| Hepatocellular Adenoma, Multiple | 1 | 7** | 9** | 7** |
| Hepatocellular Carcinoma | 4 | 28** | 22** | 20** |
| Hepatocellular Carcinoma, Multiple | 0 | 5** | 7** | 7** |
| Hepatocellular Adenoma or Carcinoma ^g | 17 | 33** | 29** | 28** |

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (15-month interim evaluation), the logistic regression test (2-year study, nonneoplastic lesions and benign neoplasms), or the life table test (2-year study malignant neoplasms and combined incidences, benign and malignant)

** $P \leq 0.01$

^a Number of animals with lesion

^b Historical incidence for 2-year NTP inhalation studies with chamber control groups at all labs (mean \pm standard deviation): 2/947 (0.2% \pm 0.7%); range, 0%-2%. Historical incidence for 2-year NTP inhalation studies with chamber control groups at Battelle Pacific Northwest Laboratories: 1/448 (0.2% \pm 0.7%); range, 0%-2%

^c Historical incidence at all labs: 12/947 (1.3% \pm 1.7%); range, 0%-6%. Historical incidence at Battelle Pacific Northwest Laboratories: 2/448 (0.5% \pm 0.9%); range, 0%-2%

^d Historical incidence at all labs: 358/947 (37.8% \pm 12.5%); range, 11%-60%. Historical incidence at Battelle Pacific Northwest Laboratories: 186/448 (41.5% \pm 9.2%); range, 30%-60%

^e Historical incidence at all labs: 1/937 (0.1% \pm 0.5%); range, 0%-2%. Historical incidence at Battelle Pacific Northwest Laboratories: 0/446

^f Historical incidence at all labs: 5/937 (0.5% \pm 1.0%); range, 0%-3%. Historical incidence at Battelle Pacific Northwest Laboratories: 2/446 (0.5% \pm 0.9%); range, 0%-2%

^g Historical incidence at all labs: 200/937 (21.3% \pm 11.9%); range, 3%-54%. Historical incidence at Battelle Pacific Northwest Laboratories: 95/446 (21.3% \pm 14.8%); range, 6%-54%

increased compared to the controls. The incidences of hepatocellular carcinoma in all exposed groups of males and females were significantly greater than those in the controls and exceeded the historical control ranges (Tables 19, C4a, and D4a).

Multiple hepatocellular carcinomas occurred in all groups of males, including controls. However, in female mice, multiple hepatocellular carcinomas were observed only in the exposed groups. The combined incidences of hepatocellular adenomas and carcinomas in exposed males and females were significantly greater than those in the controls and exceeded the historical control ranges. The incidences in males and females occurred with significant positive trends. Hepatocellular adenomas consisted of nodules of hepatocytes that compressed adjacent liver parenchyma and lacked the normal lobular and sinusoidal pattern (Plate 14). Hepatocellular carcinomas consisted of solid sheets of hepatocytes or trabeculae, three or more cells thick (Plate 15). Neoplastic hepatocytes were anaplastic, with prominent nuclei containing one or more nucleoli and variable amounts of cytoplasm. Frequently, carcinomas had multiple foci of necrosis, hemorrhage, and thrombi. Also at the end of the study, females exposed to 312 or 625 ppm had significantly greater incidences of eosinophilic foci than the controls (Tables 19 and D5).

At 15 months, coagulative multifocal necrosis of the liver occurred in one control male, one male exposed to 312 ppm, and one male exposed to 1,250 ppm (Tables 19 and C5). At the end of the study, incidences of coagulative multifocal necrosis were significantly greater in males exposed to 625 or 1,250 ppm than in the controls. Also at the end of the study, all exposed groups of females had greater incidences of

hematopoietic cell proliferation of the liver than the controls, and the increases in females exposed to 312 or 1,250 ppm were significant (Tables 19 and D5). Both coagulative multifocal necrosis and hematopoietic cell proliferation are often associated with malignant neoplasms in the liver of mice.

Hematopoietic System: At the end of the study, the incidences of histiocytic sarcoma (all organs) in all exposed groups of males and females were significantly greater than those in the controls (Tables 20, C3, and D3). The incidences of histiocytic sarcoma in all exposed groups of male and female mice exceeded the historical control ranges for all organs (Tables 20, C4b, and D4b). Although the incidences of this neoplasm were increased in exposed groups of males and females, females had higher incidences than males. The highest incidences of histiocytic sarcoma were observed in the liver and lung, and lower incidences were observed in the spleen, lymph nodes, bone marrow, and kidney. The neoplastic histiocytic cells infiltrated the liver diffusely, but sometimes formed nodular patterns (Plate 16). Marked variations in the size and shape of the neoplastic cells and nuclei and the high cytoplasmic-to-nuclear ratio were distinguishing characteristics. Neoplastic cells had abundant eosinophilic cytoplasm, and nuclei were hyperchromatic and sometimes bean shaped (Plate 17). Occasional giant cells were present. In the lung, marked perivascular infiltrates of neoplastic histiocytic cells were observed, and neoplastic cells were also present in vascular spaces. Many mice with histiocytic sarcomas had cytoplasmic eosinophilic droplets in renal tubule epithelial cells; this is consistent with other studies in which an association between renal tubule hyaline droplet and lysozyme accumulation in mice with histiocytic sarcomas has been demonstrated.

TABLE 20
Incidences of Histiocytic Sarcoma in Mice in the 2-Year Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---------------------------------------|----------------|-------------|-------------|-------------|
| Male | | | | |
| Liver ^a | 0/48 (0%) | 12/48 (25%) | 7/48 (15%) | 7/48 (15%) |
| Lung | 0/48 (0%) | 7/48 (15%) | 4/48 (8%) | 3/48 (6%) |
| Spleen | 0/48 (0%) | 2/48 (4%) | 1/46 (2%) | 2/46 (4%) |
| Mesenteric Lymph Node | 0/47 (0%) | 4/42 (10%) | 1/41 (2%) | 2/40 (5%) |
| Bone Marrow | 0/48 (0%) | 1/48 (2%) | 1/47 (2%) | 2/47 (4%) |
| Kidney | 0/48 (0%) | 3/48 (6%) | 3/48 (6%) | 2/48 (4%) |
| All Organs^b | | | | |
| Overall rate ^a | 0/48 (0%) | 12/48 (25%) | 7/48 (15%) | 7/48 (15%) |
| Adjusted rate ^c | 0.0% | 47.8% | 35.1% | 65.8% |
| Terminal rate ^d | 0/38 (0%) | 1/11 (9%) | 0/2 (0%) | 0/1 (0%) |
| First incidence (days) | — ^f | 513 | 415 | 527 |
| Life table test ^e | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test ^e | P=0.100 | P<0.001 | P=0.039 | P=0.004 |
| Female | | | | |
| Liver | 1/48 (2%) | 21/48 (44%) | 19/47 (40%) | 18/47 (38%) |
| Lung | 1/48 (2%) | 16/48 (33%) | 13/47 (28%) | 13/47 (28%) |
| Uterus | 1/48 (2%) | 0/48 (0%) | 0/45 (0%) | 0/47 (0%) |
| Spleen | 1/48 (2%) | 7/48 (15%) | 8/46 (17%) | 9/47 (19%) |
| Mesenteric Lymph Node | 1/43 (2%) | 6/40 (15%) | 7/41 (17%) | 6/43 (14%) |
| Bone Marrow | 0/48 (0%) | 6/48 (13%) | 5/46 (11%) | 4/47 (9%) |
| Kidney | 1/48 (2%) | 7/48 (15%) | 4/47 (9%) | 3/47 (6%) |
| All Organs^g | | | | |
| Overall rate | 1/48 (2%) | 21/48 (44%) | 19/47 (40%) | 18/48 (38%) |
| Adjusted rate | 2.1% | 77.6% | 51.3% | 78.2% |
| Terminal rate | 0/36 (0%) | 1/4 (25%) | 0/6 (0%) | 1/4 (25%) |
| First incidence (days) | 400 | 429 | 457 | 484 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.015 | P<0.001 | P<0.001 | P<0.001 |

^a Number of animals with neoplasm per number of animals examined microscopically

^b Historical incidence for 2-year NTP inhalation studies with chamber control groups at all labs (mean ± standard deviation): 6/950 (0.6% ± 1.2%); range, 0%-4%. Historical incidence for 2-year NTP inhalation studies with chamber controls at Battelle Pacific Northwest Laboratories: 2/450 (0.4% ± 0.9%); range, 0%-2%.

^c Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

^d Observed incidence in animals surviving until the end of the study

^e In the control column are the P values associated with the trend test. In the exposed group columns 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.

^f Not applicable; no neoplasms in animal group

^g Historical incidence at all labs: 26/941 (2.8% ± 3.1%); range, 0%-10%. Historical incidence at Battelle Pacific Northwest Laboratories: 14/447 (3.1% ± 3.0%); range, 0%-8%

Kidney: At the 15-month interim evaluation, absolute and relative kidney weights of exposed groups of male and female mice were similar to those of the controls (Table F6). Significantly increased incidences of renal tubule dilatation and karyomegaly were observed in male mice exposed to 625 or 1,250 ppm at 15 months (Tables 21 and C5). At the end of the study, the increased incidences of these lesions in exposed groups of males were generally significant. These increased incidences were considered to be related to exposure to tetrafluoroethylene. The severity of renal tubule dilatation was slightly increased at the end of the study in males exposed to 1,250 ppm. Karyomegaly was similar to that observed in the 13-week study and was most

prominent in the inner renal cortex (Plates 18 and 19). Dilated renal tubules were filled with eosinophilic amorphous material and were lined by flattened epithelium (Plates 20 and 21). Karyomegaly in renal tubule cells was characterized by nuclei that were enlarged, pleomorphic, and vesicular and had large, prominent, hyperchromatic nucleoli (Plate 20). At 15 months, karyomegaly was present in females exposed to 625 or 1,250 ppm, but at the end of the study, this lesion was observed only in females in the 1,250 ppm group. Tubule dilatation was not present in female mice at 15 months or at the end of the study. At the interim evaluation, tubule changes were more clearly

TABLE 21
Incidences of Nonneoplastic Lesions of the Kidney in Mice in the 2-Year Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|---------|-----------|------------------------|------------|
| Male | | | | |
| 15-Month Interim Evaluation | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 |
| Renal Tubule, Dilatation ^a | 0 | 0 | 6** (1.0) ^b | 10** (1.2) |
| Renal Tubule, Karyomegaly | 0 | 0 | 4* (1.0) | 10** (1.0) |
| 2-Year Study | | | | |
| Number Examined Microscopically | 48 | 48 | 48 | 48 |
| Renal Tubule, Dilatation | 0 | 4** (1.0) | 16** (1.0) | 36** (1.4) |
| Renal Tubule, Karyomegaly | 1 (1.0) | 2 (1.0) | 10** (1.0) | 28** (1.0) |
| Female | | | | |
| 15-Month Interim Evaluation | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 |
| Renal Tubule, Karyomegaly ^c | 0 | 0 | 10** (1.0) | 10** (2.0) |
| 2-Year Study | | | | |
| Number Examined Microscopically | 48 | 48 | 47 | 48 |
| Renal Tubule, Karyomegaly ^c | 0 | 0 | 0 | 38** (1.8) |

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (15-month interim evaluation) or the logistic regression test (2-year study)

** $P \leq 0.01$

^a Number of animals with lesion

^b Average severity grade of lesion in affected mice: 1=minimal; 2=mild; 3=moderate; 4=severe

^c Results of a special review

defined; however, by the end of the study, the changes were sometimes obscured by concurrent age-related background lesions (i.e., inflammation). Karyomegaly was located in the tubules in the inner renal cortex.

Spleen: Incidences of hematopoietic cell proliferation in all exposed groups of males and females were significantly greater than those in the controls at the end of the study (Tables 22, C5, and D5). Additionally, the severity of this lesion increased with increasing exposure concentration. Hematopoietic cell proliferation was frequently

associated with the presence of malignant neoplasms in the livers of affected mice.

GENETIC TOXICOLOGY

No increases in the frequency of micronucleated normochromatic erythrocytes were observed in peripheral blood samples obtained from male and female mice at the end of the 13-week inhalation study of tetrafluoroethylene (Table E1).

TABLE 22
Incidences of Nonneoplastic Lesions of the Spleen in Mice in the 2-Year Inhalation Study of Tetrafluoroethylene

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------------------|------------|------------|------------|
| Male | | | | |
| 15-Month Interim Evaluation | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 |
| Hematopoietic Cell Proliferation ^a | 2 (2.0) ^b | 4 (2.0) | 5 (1.6) | 3 (1.7) |
| 2-Year Study | | | | |
| Number Examined Microscopically | 48 | 48 | 46 | 46 |
| Hematopoietic Cell Proliferation | 14 (2.1) | 32* (2.7) | 41** (2.9) | 42** (3.0) |
| Female | | | | |
| 15-Month Interim Evaluation | | | | |
| Number Examined Microscopically | 10 | 10 | 10 | 10 |
| Hematopoietic Cell Proliferation | 2 (1.5) | 4 (1.8) | 4 (1.5) | 3 (2.0) |
| 2-Year Study | | | | |
| Number Examined Microscopically | 48 | 48 | 46 | 47 |
| Hematopoietic Cell Proliferation | 18 (2.1) | 39** (2.8) | 41** (2.9) | 41** (2.9) |

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test

** $P \leq 0.01$

^a Number of animals with lesion

^b Average severity grade of lesion in affected mice: 1=minimal; 2=mild; 3=moderate; 4=severe

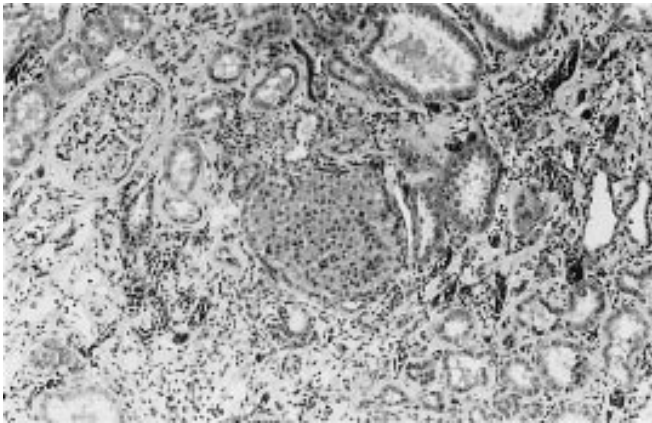


PLATE 1

Kidney of a male F344/N rat exposed to 312 ppm tetrafluoroethylene by inhalation for 2 years. Hyperplasia is focal. Note that the renal tubule is lined by increased numbers of tubule epithelial cells, which totally fill the tubule lumen. H&E; 33×

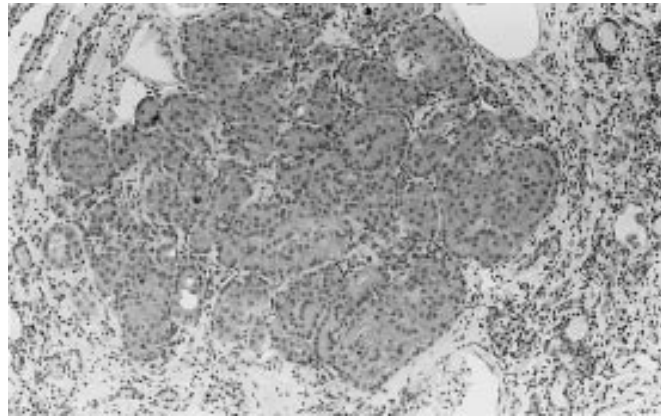


PLATE 2

Renal tubule adenoma in the kidney of a male F344/N rat exposed to 625 ppm tetrafluoroethylene by inhalation for 2 years. Note the adenoma is greater than five renal tubules in diameter and neoplastic cells are mildly pleomorphic and tend to form irregular clusters. H&E; 33×

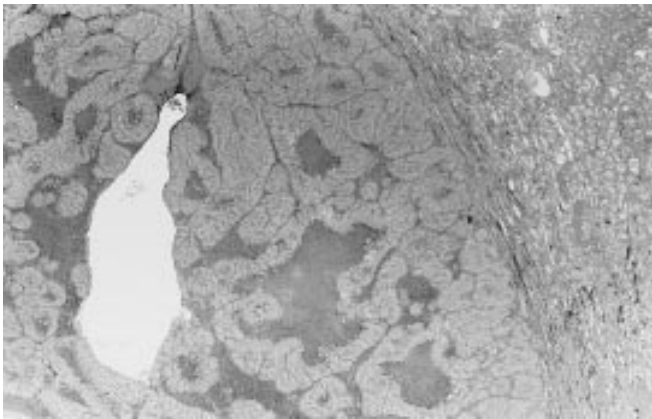


PLATE 3

Renal tubule carcinoma in the kidney of a female F344/N rat exposed to 1,250 ppm tetrafluoroethylene by inhalation for 2 years. Note the carcinoma is much larger, less discrete, and has a more prominent vascular supply than the adenoma in Plate 2. H&E; 6.6×

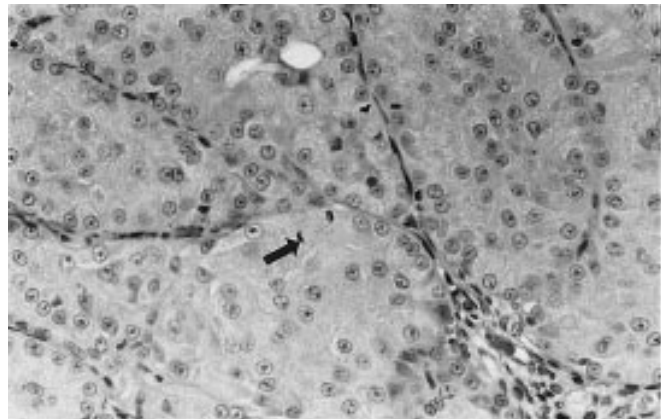


PLATE 4

Higher magnification of Plate 3. Note neoplastic cells are arranged in irregular clusters and are characterized by vesiculate nuclei with prominent nucleoli and increased mitotic figures (arrow). H&E; 100×

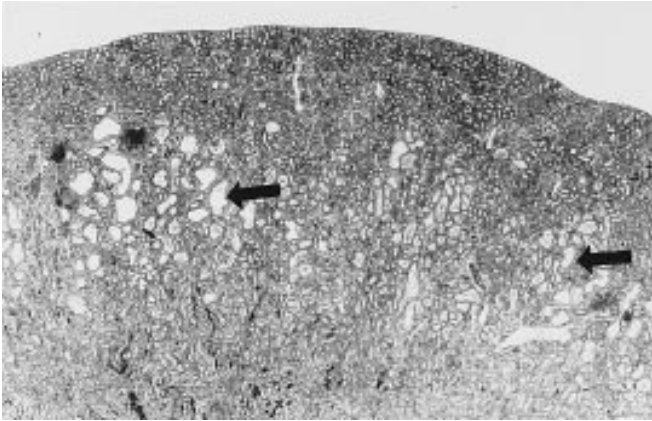


PLATE 5

Renal tubule degeneration (arrows) in a male F344/N rat exposed to 625 ppm tetrafluoroethylene by inhalation for 2 years. Degeneration is characterized by dilatation of renal tubules in the cortex near the corticomedullary junction, with or without eosinophilic debris and/or proteinaceous fluid in their lumens. H&E; 5×

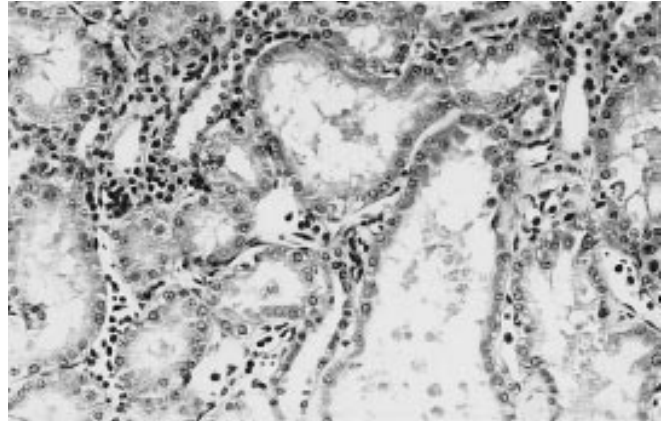


PLATE 6

Higher magnification of Plate 5 at the corticomedullary junction. Degeneration is characterized by dilatation of renal tubules with or without eosinophilic debris and/or proteinaceous fluid in their lumens. The renal tubules are lined by cuboidal epithelium. H&E; 66×

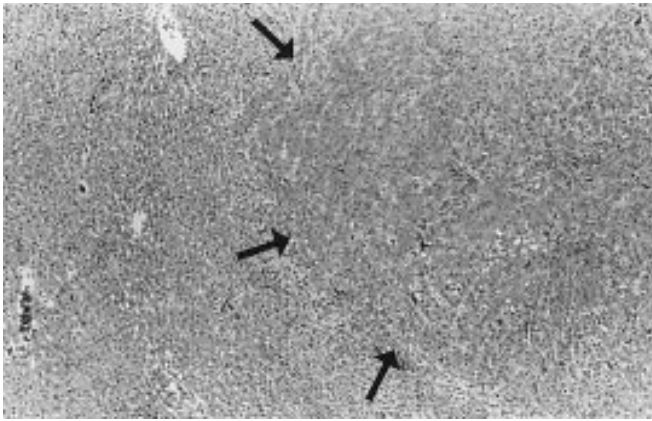


PLATE 7

Hepatocellular adenoma in the liver of a female F344/N rat exposed to 312 ppm tetrafluoroethylene by inhalation for 2 years. Note the hepatocellular adenoma caused compression of the adjacent liver parenchyma (arrows), and the adenoma lacks normal lobular architecture. H&E; 16 \times

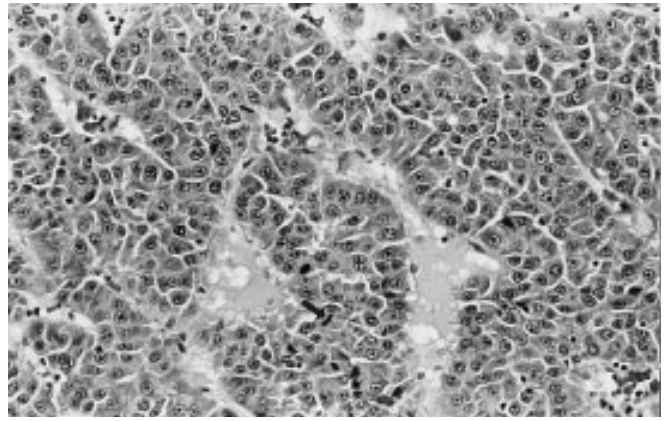


PLATE 8

Hepatocellular carcinoma from a male F344/N rat exposed to 312 ppm tetrafluoroethylene by inhalation for 2 years. The high magnification shows the trabecular pattern, three or more cells thick. Note the prominent mitotic figures (arrow). H&E; 66 \times

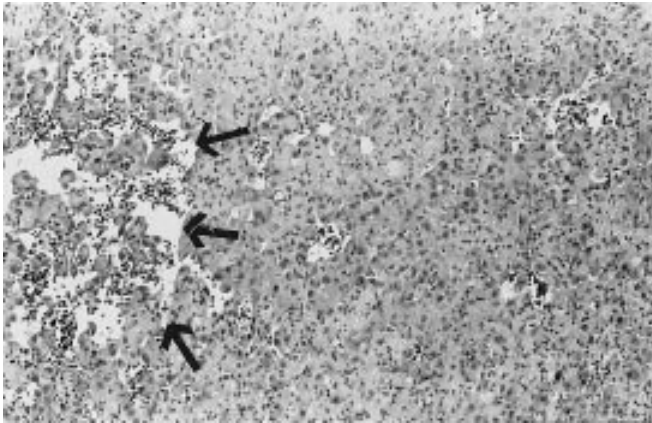


PLATE 9

Hemangiosarcoma in the liver of a female F344/N rat exposed to 625 ppm tetrafluoroethylene by inhalation for 2 years. Note the irregular, vascular spaces (arrow) containing variable numbers of erythrocytes. This hemangiosarcoma metastasized to the lung. H&E; 33 \times

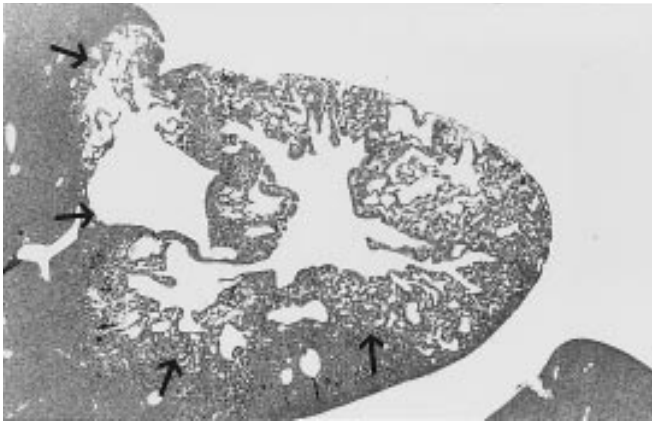


PLATE 10

Hemangioma (arrows) in the liver (L) of a male B6C3F₁ mouse exposed to 312 ppm tetrafluoroethylene by inhalation for 2 years. H&E; 6.6×

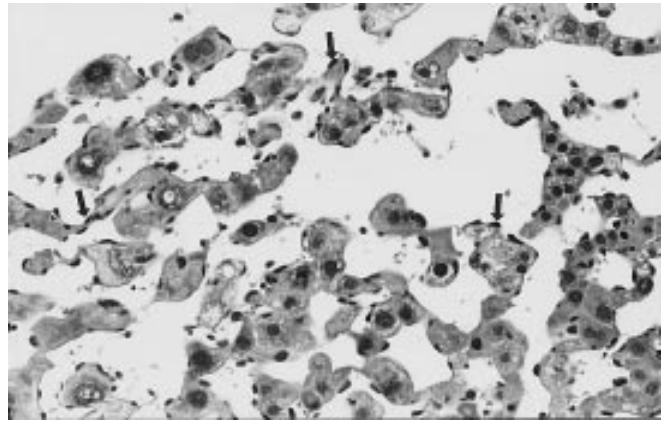


PLATE 11

Higher magnification of Plate 10 showing the dilated sinusoids with an increased number of neoplastic endothelial cells (arrows). H&E; 66×

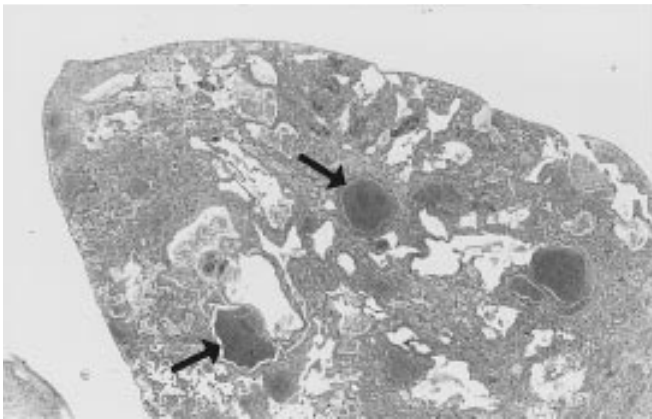


PLATE 12

Hemangiosarcoma in the liver of a male B6C3F₁ mouse exposed to 1,250 ppm tetrafluoroethylene by inhalation for 2 years. Note the large, irregular, cavernous spaces filled with erythrocytes (arrows). H&E; 5×

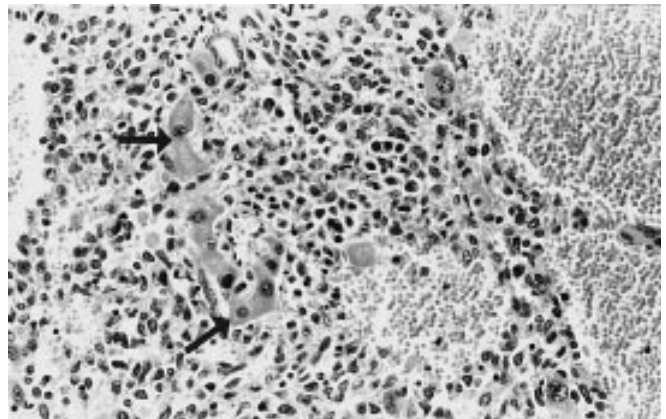


PLATE 13

Higher magnification of Plate 12, showing sheets of anaplastic, pleomorphic, endothelial cells that efface the liver parenchyma with only a few hepatocytes remaining (arrows). H&E; 80×

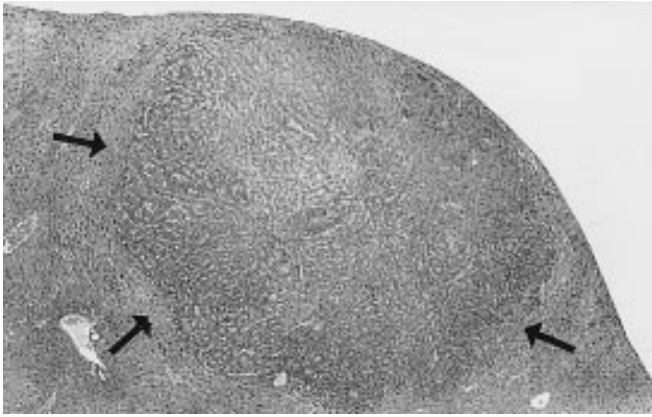


PLATE 14

Hepatocellular adenoma in the liver of a male B6C3F₁ mouse exposed to 1,250 ppm tetrafluoroethylene by inhalation for 2 years. Note the hepatocellular adenoma caused compression of the adjacent liver parenchyma (arrows), and the adenoma lacks normal lobular architecture. H&E; 10×

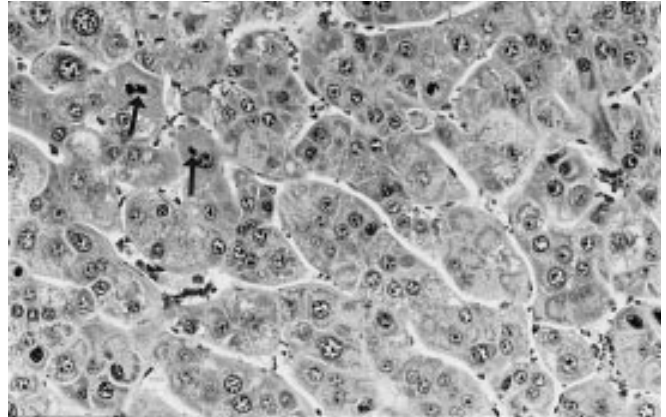


PLATE 15

Hepatocellular carcinoma from a male B6C3F₁ mouse exposed to 1,250 ppm tetrafluoroethylene by inhalation for 2 years. The high magnification shows the trabecular pattern, three or more cells thick. Note the prominent mitotic figures (arrows). H&E; 80×

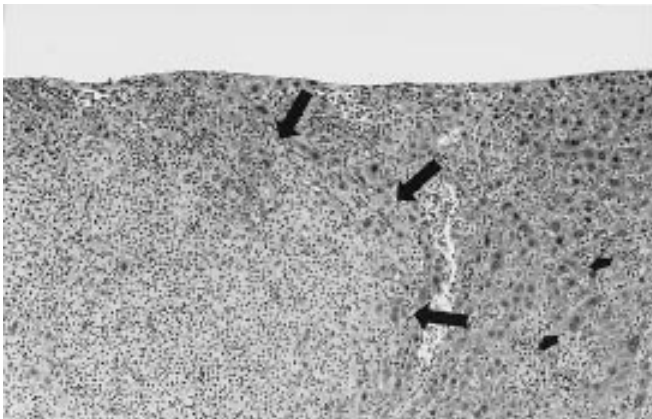


PLATE 16

Histiocytic sarcoma in the liver of a male B6C3F₁ mouse exposed to 1,250 ppm tetrafluoroethylene by inhalation for 2 years. Note the nodular (arrows) and infiltrative (arrowheads) nature of the neoplastic cells between hepatic plates. H&E; 25×

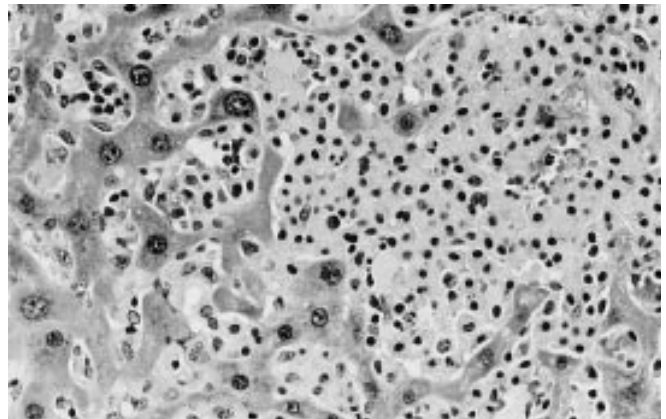


PLATE 17

Higher magnification of Plate 16. Note the neoplastic cells have abundant eosinophilic cytoplasm and nuclei are hyperchromatic and sometimes bean-shaped. H&E; 100×

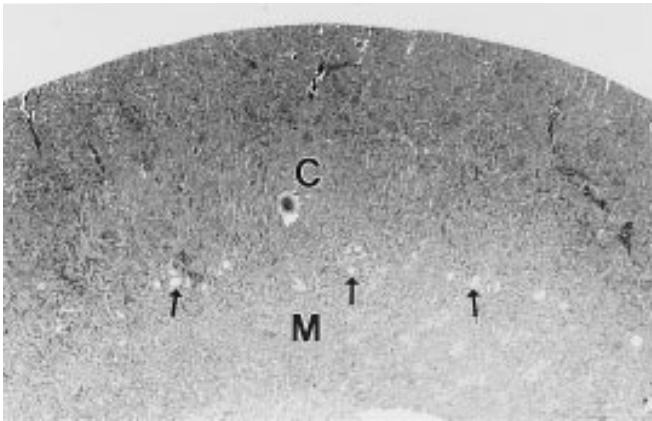


PLATE 18

Kidney of a male B6C3F₁ mouse exposed to 1,250 ppm tetrafluoroethylene for 2 years. Compare to kidney from a control mouse in Plate 19. Renal tubule dilatation (arrows) and karyomegaly were most prominent at the junction between the cortex (C) and medulla (M). H&E; 5×

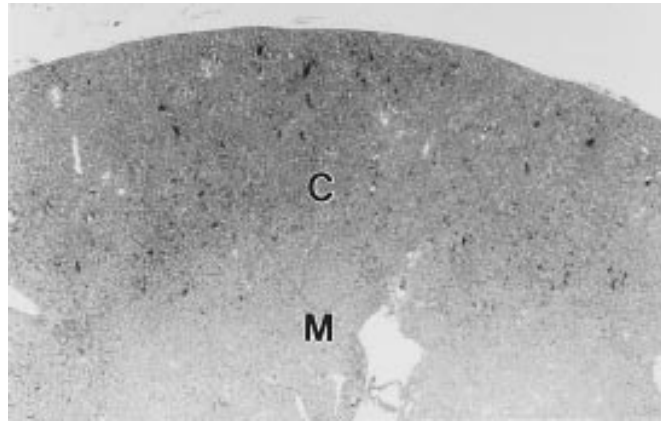


PLATE 19

Kidney from a male B6C3F₁ mouse exposed to filtered air for 2 years. Note the lack of tubular dilatation at the junction between the cortex (C) and medulla (M). H&E; 5×

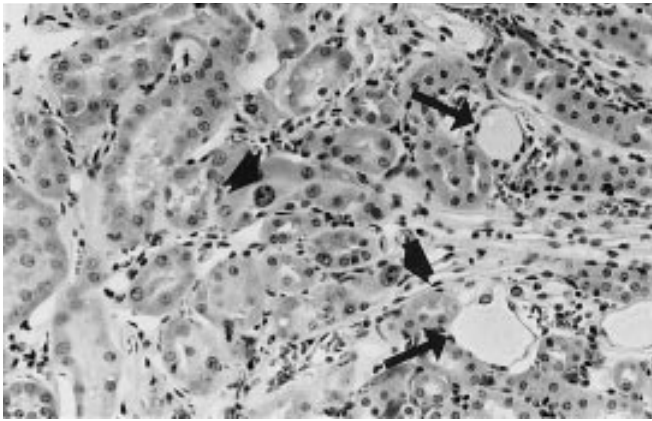


PLATE 20

Higher magnification of Plate 18 at the corticomedullary junction. Note the dilated renal tubules are filled with eosinophilic amorphous material and lined by flattened epithelium (arrows). Karyomegaly in renal tubule cells was characterized by nuclei that were enlarged and had prominent nucleoli (arrowhead). Compare with kidney from a control mouse in Plate 21. H&E; 66×

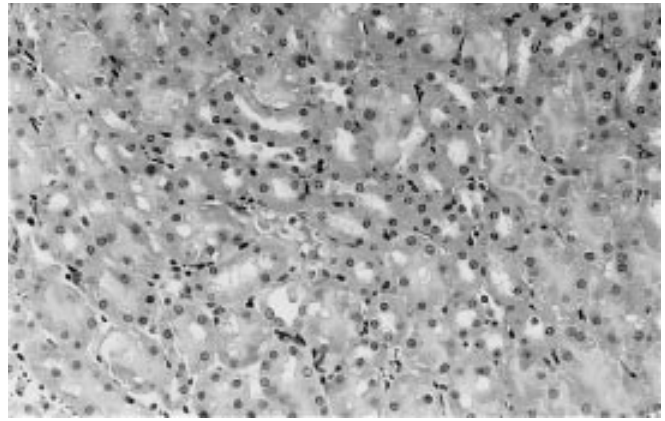


PLATE 21

Higher magnification of Plate 19. Note the lack of tubular dilatation and karyomegaly. H&E; 66×

DISCUSSION AND CONCLUSIONS

Tetrafluoroethylene was evaluated for toxicity and carcinogenicity in 16-day, 13-week, and 2-year studies in male and female F344/N rats and B6C3F₁ mice, with whole body inhalation as the route of exposure.

In the 16-day studies, there were no exposure-related deaths, clinical findings, or effects on hematologic parameters. Mean body weights of male and female rats exposed to 5,000 ppm were lower than those of the controls. Absolute kidney weights of all exposed groups of male rats, female rats exposed to 1,250 ppm, and female mice exposed to 5,000 ppm were greater than those of controls. Relative liver weights of all exposed groups of male rats and of female mice exposed to 5,000 ppm were also greater than those of the controls; however, there were no histopathologic effects in the liver. There were exposure concentration-related effects on the kidneys of exposed rats and mice, although the effects were different between the species. In rats, the kidney lesion (renal tubule degeneration) was located predominantly in the inner cortical tubule epithelial cells at the junction of the cortex and medulla and was observed in male and female rats exposed to 625 ppm or greater. This lesion has been observed previously in rats exposed to tetrafluoroethylene and other halogenated compounds and is well documented in the literature. In mice, the lesion was characterized as renal tubule karyomegaly; however, it was also located primarily in the inner renal cortex.

In the 13-week studies, there were no exposure-related deaths or clinical findings. Mean body weight gains of male and female rats exposed to 5,000 ppm were marginally reduced. Although there were no treatment-related lesions in the livers of exposed rats or mice, absolute and relative liver weights of female rats exposed to 5,000 ppm and all exposed groups of male rats were significantly increased. Absolute and relative kidney weights of male rats exposed to 1,250 ppm or greater and females exposed to 625 ppm or greater were significantly increased. Absolute and relative kidney weights of exposed mice were not significantly different from those of the controls.

Exposure concentration-related renal lesions similar to those observed in the 16-day study were observed in male rats exposed to 625 ppm or greater and in female rats in the 2,500 and 5,000 ppm groups. These lesions were considered mild at most. As with exposed rats, mice exposed to 1,250 ppm or greater in the 13-week study also developed kidney lesions similar to those observed in mice at 16 days, and these lesions were slightly more severe in males than in females. A concentration-related proteinuria occurred in all exposed groups of rats with kidney lesions. Proteinuria could be related to either glomerular or proximal tubule renal injury and, in the present study, would be consistent with the renal tubule degeneration observed morphologically. Polyuria occurred in exposed mice and probably in rats and could be related to a variety of conditions, including overdrinking, osmotic diuresis, damage to the proximal and/or distal portions of the renal tubule, and alterations of metabolism or kidney response to antidiuretic hormone (Finco, 1989). Based on an appropriate response to a water concentration test (rats only), the ability of the kidney to concentrate the urine was not compromised, indicating that the pituitary-kidney ADH axis was intact and that there was no medullary washout (which could occur with conditions such as long-term overhydration or hyperadrenocorticism). Therefore, the renal damage observed morphologically, although different for rats and mice, would appear to involve primarily the proximal convoluted tubules.

An exposure concentration-dependent normocytic, normochromic, nonresponsive anemia occurred in exposed rats and mice in the 13-week studies. Normocytic, normochromic, nonresponsive anemia has been related to selective suppression of erythropoiesis in a variety of chronic disorders and may be related to decreased erythropoietin elaboration, bone marrow suppression, or defective iron metabolism (Jain, 1986). Because there was histopathologic evidence of exposure concentration-dependent renal lesions in male and female rats and mice, the anemia would be consistent with a secondary hypoproliferative

anemia, possibly related to altered erythropoietin production/metabolism in the kidney.

In the 2-year study, survival was reduced in male rats exposed to 625 ppm and in all exposed female rats. Mean body weights of exposed rats were generally similar to those of the controls until the end of the study, at which time mean body weights were lower in males in the 625 ppm group and females in the 1,250 ppm group than in the controls. These reductions reflected the mean body weights of a very small number of surviving rats. The only exposure-related clinical finding was eye opacity in female rats, which upon histological examination was determined to be cataracts. At the 15-month interim evaluation, relative kidney weights of male rats exposed to 312 or 625 ppm and absolute kidney and liver weights of females exposed to 625 or 1,250 ppm were significantly greater than those of the controls.

The renal toxicity noted in male and female rats in the 13-week study was exacerbated in the 2-year study in that exposure to tetrafluoroethylene caused increased incidences of neoplasms and nonneoplastic lesions of the kidney in rats. As in the 13-week studies, renal tubule degeneration was associated with the proximal tubules at the corticomedullary junction, and the incidence was significantly increased at 15 months and 2 years. The only difference between the two time points was that the renal lesions were generally more severe and more numerous after 2 years.

Additional sections of the kidney were taken to further evaluate the renal proliferative effects. These sections did detect additional proliferative lesions and generally confirmed the findings of the single sections. Exposure to tetrafluoroethylene caused significantly increased incidences of hyperplasia at 15 months and 2 years in male and female rats. In the combined extended and standard evaluations, the incidences of renal tubule adenoma or carcinoma (combined) were significantly increased, with positive trends in exposed males and females. Because these neoplasms, which are considered uncommon, occurred consistently and because the incidences increased with increasing exposure concentration, the neoplasms are considered to have been caused by tetrafluoroethylene exposure. Similarly, increased incidences of renal tubule hyperplasia, adenoma, and carcinoma in male rats have been reported following inhalation exposure to 200

and 400 ppm tetrachloroethylene, a structurally related compound (NTP, 1986). In addition, incidences of karyomegaly were significantly increased in male and female rats exposed to tetrachloroethylene.

Considerable effort has been expended in the past 10 years investigating the mechanism of tetrafluoroethylene-induced renal proximal tubule toxicity, primarily in rats (Odum and Green, 1984; Boogaard *et al.*, 1989; Commandeur *et al.*, 1989; Hayden *et al.*, 1991a,b), and that induced by other halogenated compounds. Tetrafluoroethylene is conjugated in the liver with glutathione and subsequently excreted via the bile to the small intestine, where biliary and intestinal peptidases degrade them to cysteine-*S*-conjugates which may be reabsorbed from the small intestine. Glutathione conjugates released into the general circulation from the liver are also hydrolyzed to cysteine-*S*-conjugates by renal peptidases, where they are bioactivated by renal β -lyase to unstable reactive thiols. The tetrafluoroethylene cysteine conjugate, *S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine, and tetrafluoroethylene mercapturic acid have been shown to cause renal damage *in vivo* identical to that caused by tetrafluoroethylene and to have greater enzyme activation activity than several chlorinated or brominated compounds; additionally, exposure to these compounds causes their accumulation in the proximal tubule cells (Commandeur *et al.*, 1989). Like tetrafluoroethylene, they are not mutagenic. The tetrafluoroethylene cysteine conjugate has also been shown to cause death to cultured human proximal tubule cells, as indicated by the release of lactate dehydrogenase. Toxicity and binding to macromolecules in human renal cells can be prevented with aminooxyacetic acid, a known β -lyase inhibitor; this process has also been demonstrated for rat renal cells (Chen *et al.*, 1990). In rats, the cysteine conjugate is further bioactivated in the kidney and, to a lesser extent, in the liver to a difluorothionacetyl fluoride, the putative reactive metabolite for tetrafluoroethylene-induced nephrotoxicity. The thionacetyl fluorides cause mitochondrial dysfunction by inhibiting adenosine diphosphate-stimulated respiration, increasing lipid peroxidation, modifying mitochondrial proteins (HSP60 and HSP70), and inhibiting lipoyl dehydrogenase; by binding to macromolecules; and by forming protein and phospholipid adducts. The difluorothioamide adduct has been shown to cause nephrotoxicity in the

proximal tubule. Considering the similarity in effects and location of renal lesions in the present 2-year inhalation studies and the metabolism and toxicity studies reported in the literature, similar metabolites and common mechanisms may be involved in the carcinogenic response in the kidney of tetrafluoroethylene-exposed animals.

Although no liver lesions were observed in the 13-week studies, exposure to tetrafluoroethylene for 2 years caused increased incidences of nonneoplastic lesions and uncommon neoplasms of the liver in rats; these neoplasms include hemangiosarcomas, which have not been observed previously in chamber control rats from 2-year NTP inhalation studies. Hepatocellular foci (including clear cell, eosinophilic, and mixed cell) were observed in rats at the 15-month interim evaluation. At 2 years, the incidences of eosinophilic and mixed cell foci were significantly increased in exposed male rats and slightly increased in exposed female rats. At 15 months, one male exposed to 312 ppm had a hepatocellular adenoma and one male exposed to 625 ppm had a hepatocellular carcinoma. At the end of the 2-year study, male rats in the 312 ppm group had significantly increased incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined); in females, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) in all exposed groups and the incidences of hepatocellular carcinoma in the 312 and 625 ppm groups were significantly greater than those in the controls. There were no adenomas or carcinomas in control females. The incidences of hepatocellular adenoma or carcinoma (combined) occurred with positive trends in exposed males and females, even though the 8% incidence in the control male rats in the present study exceeds the previous highest incidence of 4% for inhalation chamber controls. The fact that fewer hepatocellular neoplasms occurred in male and female rats exposed to the highest concentrations (625 ppm for males, 1,250 ppm for females) than in rats exposed to lower concentrations may be a result of reduced survival. Also, the highest exposure concentration for males was half that for females.

Exposure to tetrafluoroethylene also caused hemangiosarcomas of the liver in female rats. These uncommon liver neoplasms have not been observed previously in male or female chamber controls from 2-year NTP inhalation studies or in untreated controls by any

oral route of exposure. Hepatic angiectasis, characterized by multifocal, dilated, blood-filled sinusoids with prominent lining endothelial cells, was observed in exposed groups of female rats. This lesion is sometimes associated with hepatocellular neoplasms. Cystic degeneration of the liver occurs at a low incidence in aging rats; however, following exposure of rats to hepatocarcinogens, the incidence has been shown to be increased. In fact, the incidences of cystic degeneration in exposed groups of male rats were significantly greater than the control group incidence.

The incidence of mononuclear cell leukemia in male rats exposed to 156 ppm was significantly greater than in controls. In males, interpretation of the increased incidence of mononuclear cell leukemia is complicated by reduced survival in rats exposed to 625 ppm and the fact that the control incidence exceeded the previous historical chamber control range. The incidence of mononuclear cell leukemia in male rats exposed to 312 ppm, although not significantly greater than that in the control group, also exceeded the previous historical range. Whether this response in male rats was caused by tetrafluoroethylene exposure is uncertain. However, there was a significant increase in the incidence of mononuclear cell leukemia in all exposed groups of female rats. The incidence of mononuclear cell leukemia in female controls from the present study was within the historical control range. The increased incidence of mononuclear cell leukemia in female rats is considered to have been caused by exposure to tetrafluoroethylene. Exposure to tetrachloroethylene for 2 years (NTP, 1986) also caused an increase in the incidence of mononuclear cell leukemia in male and female F344/N rats exposed to 200 or 400 ppm.

At 15 months, there were no increases in the incidence of interstitial adenoma or interstitial cell hyperplasia. At the end of the 2-year study, there was a slight but statistically significant increase in the incidence of interstitial cell adenoma in the testes of rats exposed to 312 or 625 ppm. It is uncertain whether these increased incidences of adenomas were due to tetrafluoroethylene exposure; however, there was a similar slight but significant increase in the incidence of interstitial cell adenomas in F344/N rats exposed to 200 or 400 ppm tetrachloroethylene for 2 years (NTP, 1986).

Exposure to tetrafluoroethylene for 2 years caused a significant increase in the incidence of cataracts in female rats exposed to 1,250 ppm. The lesion was characterized by a disruption of the normal organization of lens fibers, with swelling, vacuolization, and mineralization.

In the 2-year mouse study, survival was reduced in all exposed male and female mice to the extent that the study was terminated at week 96. The reduction in survival was considered to be the result of exposure-related neoplasms of the liver. Mean body weights of exposed mice were generally similar to those of controls except at the end of the study, where survival was significantly decreased.

As observed in the 2-year rat study, exposure of mice to tetrafluoroethylene caused significant increases in the incidences of nonneoplastic lesions and uncommon neoplasms of the liver in mice at 15 months and 2 years. Exposure to tetrafluoroethylene caused hemangiomas in the liver of male and female mice, and multiple hemangiomas were observed in males and females. These uncommon neoplasms were not observed in control mice. Hemangiosarcomas caused by exposure to tetrafluoroethylene were observed as early as 15 months in males in the 1,250 ppm group and females in the 312 ppm group. Hemangiosarcomas occurred with statistically significant incidences and with a positive trend in all groups of exposed males and females. The incidences of multiple hemangiosarcomas were significant in all exposed groups of males and females. In a few males and females, the hemangiosarcomas metastasized to the lung. Hemangiosarcomas were not observed in male or female control mice. The incidences of hemangioma or hemangiosarcoma (combined) were also highly significant and occurred with a positive trend for exposed groups of males and females. Hemangiomas and hemangiosarcomas are uncommon neoplasms in mice in studies using such routes as feed or dosed water, and the historical incidences of these neoplasms in untreated mice from 2-year NTP feed and dosed water studies are similar to those for inhalation chamber control mice. The rarity of these neoplasms further accentuates the striking results of the present study. These uncommon neoplasms probably had a significant effect on the survival of mice in the present study.

Although exposure to 100 or 200 ppm tetrachloroethylene for 2 years caused increased incidences of hepatocellular adenoma and carcinoma in male and female mice, there were no exposure-related increases in hemangioma or hemangiosarcoma incidences (NTP, 1986). However, chronic inhalation exposure to vinyl chloride (chloroethylene) has caused hemangiosarcomas and hepatocellular adenomas and carcinomas in several strains of rats, mice, and hamsters (IARC, 1979, 1987). More importantly, a number of epidemiologic studies have shown that workers exposed to vinyl chloride have higher mortality rates and a high incidence of neoplasms in the liver, brain, lung, and lymphatic and hematopoietic systems, all attributed to vinyl chloride exposure. Neoplasms observed in the liver of these workers include hemangiosarcomas and hepatocellular adenomas and carcinomas. Liver neoplasms caused by vinyl chloride exposure in laboratory animals and humans are similar to those observed in rats and mice exposed to tetrafluoroethylene.

Angiectasis, eosinophilic foci, and coagulative multifocal necrosis of the liver were observed at 15 months and were significantly increased in incidence and severity at 2 years. The incidences of hepatocellular adenoma or carcinoma (combined) were increased in all exposed groups of male mice and were significantly greater in all exposed groups of female mice than in the control group. Many animals had multiple adenomas or carcinomas. In all exposed groups, the incidences of multiple adenomas and multiple carcinomas in female mice and multiple carcinomas in male mice were significantly increased. A number of the carcinomas in exposed males and females, but only one carcinoma in each of the male and female control groups, metastasized to the lung. Incidences of hepatocellular adenoma, carcinoma, and adenoma or carcinoma (combined) in control male and female mice were within the historical ranges in chamber control mice from 2-year NTP inhalation studies. The increased incidences of these neoplasms in exposed male and female mice were considered to be exposure-related. A low frequency (15%) of *H-ras* codon 61 mutations was observed in tetrafluoroethylene-induced hepatocellular neoplasms when compared to the frequency in neoplasms in controls (59%) or in spontaneous liver neoplasms of B6C3F₁ mice (56%) (Appendix L). This frequency is similar to that detected in liver neoplasms in B6C3F₁ mice exposed to

tetrachloroethylene (24%) (Anna *et al.*, 1994). Although a few neoplasms in the tetrachloroethylene study were found to have a *K-ras* mutation, none were found in neoplasms in the tetrafluoroethylene study. These data indicate that these two hepatocellular carcinogens induce liver neoplasms via a *ras*-independent pathway.

The incidence of histiocytic sarcoma was markedly increased in exposed male mice and significantly increased in all female mice, although not in an exposure concentration-related manner. Among mice with any histiocytic sarcomas, histiocytic sarcomas were present in the liver of all animals and in the lung of many animals. The spleen, lymph nodes, bone marrow, and kidney were also affected. Combined with the hepatocellular adenomas and carcinomas, hemangiomas, and hemangiosarcomas, the presence of histiocytic sarcomas in exposed mice compromise liver function, ultimately leading to the death of the mice.

In the 13-week mouse study, renal tubule karyomegaly, accompanied by a polyuria, occurred in male and female mice exposed to 1,250 ppm or greater and was the only consideration for exposure concentration selection for the 2-year study. Although there were no kidney neoplasms in exposed groups of mice as were observed in the 2-year rat study, there were significantly increased incidences of renal tubule dilatation in exposed males and renal tubule karyomegaly, located in the tubules in the inner cortex, in males and females. These lesions were observed at 15 months as well as at the end of the study. In general, only the incidences of these lesions increased over the course of the study, while the severities remained essentially unchanged; in fact, the severity of karyomegaly was similar to that observed in the 13-week study at the same exposure concentration. Although these lesions were observed in all exposed groups of males at the end of the study, incidences of karyomegaly were observed only in 1,250 ppm females; however, this group had a high incidence. As in the 13-week study, males were affected more than

females, and the increased incidences of both nonneoplastic lesions were considered to be related to exposure to tetrafluoroethylene. The effect of tetrafluoroethylene exposure on the kidney of mice is very similar to the effects of exposure to 100 or 200 ppm tetrachloroethylene for 2 years (NTP, 1986), in that tetrachloroethylene caused increased incidences of karyomegaly in male and female mice without causing increased incidences of kidney neoplasms.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of tetrafluoroethylene in male F344/N rats based on increased incidences of renal tubule neoplasms (mainly adenomas) and hepatocellular neoplasms. There was *clear evidence of carcinogenic activity* of tetrafluoroethylene in female F344/N rats based on increased incidences of renal tubule neoplasms, liver hemangiosarcomas, hepatocellular neoplasms, and mononuclear cell leukemia. There was *clear evidence of carcinogenic activity* of tetrafluoroethylene in male and female B6C3F₁ mice based on increased incidences of liver hemangiomas and hemangiosarcomas, hepatocellular neoplasms, and histiocytic sarcomas.

Slight increases in the incidences of mononuclear cell leukemia and testicular interstitial cell adenomas in male rats may have been related to exposure to tetrafluoroethylene.

Exposure of rats to tetrafluoroethylene resulted in increased incidences of renal tubule hyperplasia and degeneration in males and females, increased severity of kidney nephropathy in males, and increased incidences of liver angiectasis and cataracts in females. Exposure of mice to tetrafluoroethylene resulted in increased incidences of hematopoietic cell proliferation of the liver in females, liver angiectasis in males and females, renal tubule dilatation in males, renal tubule karyomegaly in males and females, and splenic hematopoietic cell proliferation in males and females.

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

REFERENCES

- Anna, C.H., Maronpot, R.R., Pereira, M.A., Foley, J.F., Malarkey, D.E., and Anderson, M.W. (1994). *ras* Proto-oncogene activation in dichloroacetic acid-, trichloroethylene- and tetrachloroethylene-induced liver tumors in B6C3F1 mice. *Carcinogenesis* **15**, 2255-2261.
- Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 362-365. John Wiley and Sons, New York.
- Boogaard, P.J., Commandeur, J.N.M., Mulder, G.J., Vermeulen, N.P.E., and Nagelkerke, J.F. (1989). Toxicity of the cysteine *S*-conjugates and mercapturic acids of four structurally related difluoroethylenes in isolated proximal tubular cells from rat kidney. Uptake of the conjugates and activation to toxic metabolites. *Biochem. Pharmacol.* **38**, 3731-3741.
- 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.
- Bretherick, L. (1985). *Handbook of Reactive Chemical Hazards*, 3rd ed., p. 1348. Butterworths, Boston, MA.
- Bruschi, S.A., West, K.A., Crabb, J.W., Gupta, R.S., and Stevens, J.L. (1993). Mitochondrial HSP60 (P1 protein) and a HSP70-like protein (Mortalin) are major targets for modification during *S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine-induced nephrotoxicity. *J. Biol. Chem.* **268**, 23,157-23,161.
- Chen, J.C., Stevens, J.L., Trifillis, A.L., and Jones, T.W. (1990). Renal cysteine conjugate β -lyase-mediated toxicity studied with primary cultures of human proximal tubular cells. *Toxicol. Appl. Pharmacol.* **103**, 463-473.
- Chen, Q., Yu, K., and Stevens, J.L. (1992). Regulation of the cellular stress response by reactive electrophiles. The role of covalent binding and cellular thiols in transcriptional activation of the 70-kilodalton heat shock protein gene by nephrotoxic cysteine conjugates. *J. Biol. Chem.* **267**, 24,322-24,327.
- Code of Federal Regulations (CFR) **21**, Part 58.
- Commandeur, J.N.M., Brakenhoff, J.P.G., de Kanter, F.J.J., and Vermeulen, N.P.E. (1988). Nephrotoxicity of mercapturic acids of three structurally related 2,2-difluoroethylenes in the rat. *Biochem. Pharmacol.* **37**, 4495-4504.
- Commandeur, J.N.M., de Kanter, F.J.J., and Vermeulen, N.P.E. (1989). Bioactivation of the cysteine-*S*-conjugate and mercapturic acid of tetrafluoroethylene to acylating reactive intermediates in the rat: Dependence of activation and deactivation activities on acetyl coenzyme A availability. *Mol. Pharmacol.* **36**, 654-663.
- Commandeur, J.N.M., Stijntjes, G.J., Wijngaard, J., and Vermeulen, N.P.E. (1991). Metabolism of *L*-cysteine *S*-conjugates and *N*-(trideuteroacetyl)-*L*-cysteine *S*-conjugates of four fluoroethylenes in the rat. Role of balance of deacetylation and acetylation in relation to the nephrotoxicity of mercapturic acids. *Biochem. Pharmacol.* **42**, 31-38.

- Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.
- Dilley, J.V., Carter, V.L., Jr., and Harris, E.S. (1974). Fluoride ion excretion by male rats after inhalation of one of several fluoroethylenes or hexafluoropropene. *Toxicol. Appl. Pharmacol.* **27**, 582-590.
- Ding, X.Z., Yu, H.T., Hu, M., Liu, C.F., and Ko, F.Z. (1980). Studies on the absorption, distribution, and elimination of four organofluorine compounds in rabbits. *Chung Hua Yu Fang I Hsueh Tsa Chih* **14**, 39-42.
- 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.
- Dixon, W.J., and Massey, F.J., Jr. (1951). *Introduction to Statistical Analysis*, 1st ed., pp. 145-147. McGraw-Hill Book Company, New York.
- Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.
- Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.
- Finco, D.R. (1989). Kidney function. In *Clinical Biochemistry of Domestic Animals* (J.J. Kaneko, Ed.), 4th ed., pp. 496-542. Academic Press, Inc., San Diego, CA.
- Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* **10** (Suppl. 10), 1-175.
- Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *J. Natl. Cancer Inst.* **62**, 957-974.
- Gosselin, R.E., Hodge, H.C., Smith, R.P., and Gleason, M.N. (1984). *Clinical Toxicology of Commercial Products: Acute Poisoning*, Vol. II, p. 412. Williams and Wilkins, Baltimore, MD.
- Green, T., and Odum, J. (1985). Structure/activity studies of the nephrotoxic and mutagenic action of cysteine conjugates of chloro- and fluoro-alkenes. *Chem.-Biol. Interact.* **54**, 15-31.
- Groves, C.E., Hayden, P.J., Lock, E.A., and Schnellmann, R.G. (1993). Differential cellular effects in the toxicity of haloalkene and haloalkane cysteine conjugates to rabbit renal proximal tubules. *J. Biochem. Toxicol.* **8**, 49-56.
- Harris, J.W., Dekant, W., and Anders, M.W. (1992). *In vivo* detection and characterization of protein adducts resulting from bioactivation of haloethene cysteine S-conjugates by ¹⁹F NMR: Chlorotri-fluoroethene and tetrafluoroethene. *Chem. Res. Toxicol.* **5**, 34-41.
- Haseman, J.K. (1984). Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* **58**, 385-392.
- Hawley, G.G., Ed. (1981). *The Condensed Chemical Dictionary*, 10th ed. Van Nostrand Reinhold, New York.
- Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., and Zeiger, E. (1983). Salmonella mutagenicity test results for 250 chemicals. *Environ. Mutagen.* **5** (Suppl. 1), 3-142.
- Hayden, P.J., and Stevens, J.L. (1990). Cysteine conjugate toxicity, metabolism, and binding to macromolecules in isolated rat kidney mitochondria. *Mol. Pharmacol.* **37**, 468-476.

- Hayden, P.J., Ichimura, T., McCann, D.J., Pohl, L.R., and Stevens, J.L. (1991a). Detection of cysteine conjugate metabolite adduct formation with specific mitochondrial proteins using antibodies raised against halothane metabolite adducts. *J. Biol. Chem.* **266**, 18,415-18,418.
- Hayden, P.J., Yang, Y., Ward, A.J.I., Dulik, D.M., McCann, D.J., and Stevens, J.L. (1991b). Formation of difluorothionacetyl-protein adducts by S-(1,1,2,2-tetrafluoroethyl)-L-cysteine metabolites: Nucleophilic catalysis of stable lysyl adduct formation by histidine and tyrosine. *Biochemistry* **30**, 5935-5943.
- Hayden, P.J., Welsh, C.J., Yang, Y., Schaefer, W.H., Ward, A.J.L., and Stevens, J.L. (1992). Formation of mitochondrial phospholipid adducts by nephrotoxic cysteine conjugate metabolites. *Chem. Res. Toxicol.* **5**, 231-237.
- Hazardous Substances Data Base (HSDB) (1995). Maintained, reviewed, and updated on the National Library of Medicine's Toxicology Data Network (TOXNET). Available through the Medlars System.
- Hollander, M., and Wolfe, D.A. (1973). *Nonparametric Statistical Methods*, pp. 120-123. John Wiley and Sons, New York.
- International Agency for Research on Cancer (IARC) (1979). Some Monomers, Plastics and Synthetic Elastomers, and Acrolein. In *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. Vol. 19, pp. 285-301. Lyon, France.
- International Agency for Research on Cancer (IARC) (1987). Overall Evaluations of Carcinogenicity: An Updating of *IARC Monographs*. In *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. Vols. 1-42, Suppl. 7. Lyon, France.
- Jain, N.C., Ed. (1986). Depression or hypoproliferative anemias. In *Schalm's Veterinary Hematology*, 4th ed., pp. 655-675. Lea and Febiger, Philadelphia, PA.
- 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.
- Kennedy, G.L. (1990). Toxicology of fluorine-containing monomers. In *Critical Reviews in Toxicology*, Vol. 21, pp. 149-170. CRC Press, Boca Raton, FL.
- Lock, E.A., and Schnellmann, R.G. (1990). The effect of haloalkene cysteine conjugates on rat renal glutathione reductase and lipoyl dehydrogenase activities. *Toxicol. Appl. Pharmacol.* **104**, 180-190.
- 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.
- MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.
- 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.
- Material Safety Data Sheet Collection* (1993). Tetrafluoroethylene. Sheet No. 851. Genium Publishing Corp., Schenectady, NY.
- Morrison, D.F. (1976). *Multivariate Statistical Methods*, 2nd ed., pp. 170-179. McGraw-Hill Book Company, New York.
- Myhr, B., McGregor, D., Bowers, L., Riach, C., Brown, A.G., Edwards, I., McBride, D., Martin, R., and Caspary, W.J. (1990). L5178Y mouse lymphoma cell mutation assay results with 41 compounds. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 138-167.

- National Cancer Institute (NCI) (1976). Guidelines for Carcinogen Bioassay in Small Rodents. Technical Report Series No. 1. NIH Publication No. 76-801. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.
- National Cancer Institute (NCI) (1977). Bioassay of Tetrachloroethylene for Possible Carcinogenicity. (CAS No. 127-18-4). Technical Report Series No. 13. NIH Publication No. 77-813. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD.
- National Fire Protection Association (NFPA) (1986). Fire Protection Guide on Hazardous Materials. 9th ed., Sect. 49, pp. 86-87. National Fire Protection Association, Quincy, MA.
- National Institute for Occupational Safety and Health (NIOSH) (1990). National Occupational Exposure Survey (1981-1983), unpublished provisional data as of July 1, 1990. NIOSH, Cincinnati, OH.
- National Institutes of Health (NIH) (1978). Open Formula Rat and Mouse Ration (NIH-07). Specification NIH-11-1335. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.
- National Library of Medicine (1995). MEDLINE, CANCERLIT, TOXLINE. Toxicology Data Bank databases maintained by the National Library of Medicine. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD.
- National Toxicology Program (NTP) (1984). Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluations in Toxicity Testing for Rats and Mice, 10/31/82 version (updated October 1984). Research Triangle Park, NC.
- National Toxicology Program (NTP) (1986). NTP Technical Report on the Toxicology and Carcinogenesis of Tetrachloroethylene (Perchloroethylene) (CAS No. 127-18-4) in F344/N Rats and B6C3F₁ Mice (Inhalation Studies). Technical Report Series No. 311. NIH Publication No. 86-2567. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park.
- Odum, J., and Green, T. (1984). The metabolism and nephrotoxicity of tetrafluoroethylene in the rat. *Toxicol. Appl. Pharmacol.* **76**, 306-318.
- Patty's Industrial Hygiene and Toxicology* (1994). 4th ed., Vol. II (G.D. Clayton and F.E. Clayton, Eds.), p. 1149. Wiley-Interscience, New York.
- Sakharova, L.N., and Tolgskaya, M.S. (1977). Toxicity and the nature of action of some halogen derivatives of ethylene, such as difluorodichloroethylene, trifluorochloroethylene and tetrafluoroethylene. *Gig. Tr. Prof. Zabol.* **21**, Vol. 5, 36-42.
- Schmid, W. (1976). The micronucleus test for cytogenetic analysis. In *Chemical Mutagens, Principles and Methods for Their Detection* (A. Hollaender, Ed.), Vol. 4, pp. 31-53. Plenum Press, New York.
- Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.
- Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.
- U.S. International Trade Commission (USITC) (1994). Synthetic Organic Chemicals, United States Production and Sales, 1992. USITC Publication No. 2720. USITC, Washington, DC.
- Weast, R.C., Ed. (1985). *CRC Handbook of Chemistry and Physics*, 66th ed., p. C272. CRC Press, Boca Raton, FL.
- 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.
- Zhemerdei, A.I. (1958). Toxicology of tetrafluoroethylene. *Tr. Leningr. Sanit. Gig. Med. Inst.* **44**, 164.

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

| | | |
|------------------|---|------------|
| TABLE A1 | Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Tetrafluoroethylene | 81 |
| TABLE A2 | Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Tetrafluoroethylene | 86 |
| TABLE A3 | Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Tetrafluoroethylene | 108 |
| TABLE A4a | Historical Incidence of Renal Tubule Neoplasms in Chamber Control Male F344/N Rats | 115 |
| TABLE A4b | Historical Incidence of Hepatocellular Neoplasms in Chamber Control Male F344/N Rats | 115 |
| TABLE A4c | Historical Incidence of Mononuclear Cell Leukemia in Chamber Control Male F344/N Rats | 116 |
| TABLE A5 | Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Tetrafluoroethylene | 117 |

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Tetrafluoroethylene ^a

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|---------------------------------------|---------|---------|-----------|---------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation | 10 | 10 | 10 | 10 |
| Early deaths | | | | |
| Moribund | 27 | 34 | 32 | 48 |
| Natural deaths | 6 | 4 | 1 | 1 |
| Survivors | | | | |
| Terminal sacrifice | 17 | 12 | 17 | 1 |
| Animals examined microscopically | 60 | 60 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Intestine large, colon | (10) | (10) | (10) | (10) |
| Polyp adenomatous | | 1 (10%) | | |
| Intestine small, duodenum | (10) | (10) | (10) | (10) |
| Liver | (10) | (10) | (10) | (10) |
| Hepatocellular carcinoma | | | | 1 (10%) |
| Hepatocellular adenoma | | | 1 (10%) | |
| Mesentery | (2) | | (2) | (4) |
| Endocrine System | | | | |
| Pituitary gland | (10) | (9) | (10) | (10) |
| Pars distalis, adenoma | 2 (20%) | 3 (33%) | 1 (10%) | 4 (40%) |
| Pars distalis, adenoma, multiple | 1 (10%) | | | |
| Pars intermedia, adenoma | 1 (10%) | | | |
| Thyroid gland | (10) | (10) | (10) | (10) |
| Follicular cell, adenoma | 1 (10%) | | | |
| Genital System | | | | |
| Epididymis | (10) | (10) | (10) | (10) |
| Preputial gland | (10) | (10) | (10) | (10) |
| Carcinoma | 1 (10%) | | | |
| Testes | (10) | (10) | (10) | (10) |
| Bilateral, interstitial cell, adenoma | 7 (70%) | 5 (50%) | 10 (100%) | 9 (90%) |
| Interstitial cell, adenoma | 2 (20%) | 2 (20%) | | 1 (10%) |
| Integumentary System | | | | |
| Skin | (10) | (10) | (10) | (10) |
| Subcutaneous tissue, sarcoma | | | 1 (10%) | |
| Respiratory System | | | | |
| Lung | (10) | (10) | (10) | (10) |
| Alveolar/bronchiolar carcinoma | 1 (10%) | | | |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--|----------|----------|----------|---------|
| 15-Month Interim Evaluation (continued) | | | | |
| Urinary System | | | | |
| Kidney | (10) | (10) | (10) | (10) |
| Renal tubule, carcinoma | | | | 1 (10%) |
| Urinary bladder | (10) | (10) | (10) | (10) |
| Transitional epithelium, papilloma | | 1 (10%) | | |
| Systemic Lesions | | | | |
| Multiple organs ^b | (10) | (10) | (10) | (10) |
| Leukemia mononuclear | | | | 1 (10%) |
| Mesothelioma malignant | | | | 1 (10%) |
| Systems Examined With No Neoplasms Observed | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| Hematopoietic System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |
| Special Senses System | | | | |
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Intestine large, colon | (50) | (50) | (50) | (50) |
| Intestine large, cecum | (50) | (50) | (50) | (50) |
| Intestine small, duodenum | (50) | (50) | (50) | (50) |
| Intestine small, jejunum | (48) | (47) | (49) | (50) |
| Intestine small, ileum | (49) | (49) | (50) | (50) |
| Liver | (50) | (50) | (50) | (50) |
| Hepatocellular carcinoma | 1 (2%) | 1 (2%) | 10 (20%) | 3 (6%) |
| Hepatocellular adenoma | 2 (4%) | 3 (6%) | 5 (10%) | 4 (8%) |
| Hepatocellular adenoma, multiple | 1 (2%) | 3 (6%) | 3 (6%) | 1 (2%) |
| Hepatocholangioma | | 1 (2%) | | |
| Mesentery | (15) | (11) | (18) | (14) |
| Hepatocellular carcinoma, metastatic, liver | | | | 1 (7%) |
| Pancreas | (50) | (50) | (50) | (50) |
| Hepatocellular carcinoma, metastatic, liver | | | | 1 (2%) |
| Acinus, adenoma | | 2 (4%) | 1 (2%) | 1 (2%) |
| Pharynx | (1) | | | |
| Palate, squamous cell carcinoma | 1 (100%) | | | |
| Salivary glands | (50) | (50) | (50) | (50) |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Stomach, glandular | (50) | (50) | (50) | (50) |
| Tongue | (1) | (1) | | (1) |
| Squamous cell papilloma | 1 (100%) | 1 (100%) | | |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|---------------------------------------|----------|----------|----------|----------|
| 2-Year Study (continued) | | | | |
| Cardiovascular System | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Schwannoma NOS | | 1 (2%) | | |
| Endocrine System | | | | |
| Adrenal cortex | (50) | (50) | (50) | (50) |
| Adenoma | | | 2 (4%) | |
| Adrenal medulla | (50) | (50) | (50) | (50) |
| Ganglioneuroma | | | | 1 (2%) |
| Pheochromocytoma malignant | 2 (4%) | | 1 (2%) | |
| Pheochromocytoma complex | | 1 (2%) | | |
| Pheochromocytoma benign | 15 (30%) | 8 (16%) | 8 (16%) | 6 (12%) |
| Bilateral, pheochromocytoma benign | 4 (8%) | 3 (6%) | 1 (2%) | 4 (8%) |
| Islets, pancreatic | (50) | (50) | (50) | (50) |
| Adenoma | 3 (6%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Carcinoma | 1 (2%) | 5 (10%) | 1 (2%) | 1 (2%) |
| Parathyroid gland | (50) | (50) | (50) | (49) |
| Pituitary gland | (50) | (49) | (49) | (49) |
| Pars distalis, adenoma | 39 (78%) | 37 (76%) | 35 (71%) | 23 (47%) |
| Pars intermedia, adenoma | 1 (2%) | | | |
| Thyroid gland | (50) | (50) | (50) | (50) |
| C-cell, adenoma | 7 (14%) | 8 (16%) | 5 (10%) | 4 (8%) |
| C-cell, carcinoma | 2 (4%) | 1 (2%) | 4 (8%) | 1 (2%) |
| Follicular cell, adenoma | 1 (2%) | | 1 (2%) | |
| General Body System | | | | |
| None | | | | |
| Genital System | | | | |
| Epididymis | (50) | (50) | (50) | (50) |
| Preputial gland | (50) | (50) | (50) | (49) |
| Adenoma | 1 (2%) | 3 (6%) | 5 (10%) | 2 (4%) |
| Carcinoma | 3 (6%) | 3 (6%) | 1 (2%) | 4 (8%) |
| Bilateral, carcinoma | | | 1 (2%) | |
| Prostate | (50) | (50) | (50) | (50) |
| Seminal vesicle | (50) | (50) | (50) | (50) |
| Testes | (50) | (50) | (50) | (50) |
| Bilateral, interstitial cell, adenoma | 35 (70%) | 37 (74%) | 40 (80%) | 42 (84%) |
| Interstitial cell, adenoma | 4 (8%) | 3 (6%) | 8 (16%) | 5 (10%) |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Lymph node | (11) | (17) | (16) | (17) |
| Lymph node, bronchial | (45) | (50) | (46) | (47) |
| Lymph node, mandibular | (50) | (49) | (49) | (46) |
| Lymph node, mesenteric | (50) | (50) | (50) | (50) |
| Lymph node, mediastinal | (49) | (47) | (48) | (46) |
| Spleen | (50) | (50) | (50) | (50) |
| Thymus | (49) | (47) | (47) | (45) |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--|--------|---------|----------|---------|
| 2-Year Study (continued) | | | | |
| Integumentary System | | | | |
| Mammary gland | (33) | (36) | (41) | (34) |
| Adenoma | | | | 1 (3%) |
| Carcinoma | | 1 (3%) | 1 (2%) | |
| Fibroadenoma | 2 (6%) | 2 (6%) | 2 (5%) | 1 (3%) |
| Skin | (49) | (50) | (50) | (50) |
| Basosquamous tumor benign | 2 (4%) | | | 1 (2%) |
| Keratoacanthoma | 3 (6%) | | 1 (2%) | 1 (2%) |
| Squamous cell carcinoma | 1 (2%) | | 1 (2%) | |
| Squamous cell papilloma | 1 (2%) | | | 1 (2%) |
| Subcutaneous tissue, fibroma | 3 (6%) | 1 (2%) | | 1 (2%) |
| Subcutaneous tissue, fibrosarcoma | | | 1 (2%) | 1 (2%) |
| Subcutaneous tissue, hemangiosarcoma | | | 1 (2%) | |
| Subcutaneous tissue, neurofibroma | 1 (2%) | | 1 (2%) | |
| Subcutaneous tissue, neurofibrosarcoma | | | 1 (2%) | |
| Subcutaneous tissue, sarcoma | | | | 1 (2%) |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Osteosarcoma | | 1 (2%) | | |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Meningioma NOS | | | 1 (2%) | |
| Respiratory System | | | | |
| Larynx | (50) | (50) | (50) | (50) |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | | 1 (2%) | 2 (4%) | 1 (2%) |
| Carcinoma, metastatic, kidney | 1 (2%) | | | |
| Chordoma, metastatic, uncertain primary site | | 1 (2%) | | 1 (2%) |
| Hepatocellular carcinoma, metastatic, liver | | | 1 (2%) | |
| Nose | (50) | (50) | (50) | (50) |
| Trachea | (50) | (50) | (50) | (50) |
| Special Senses System | | | | |
| Zymbal's gland | | | (1) | |
| Carcinoma | | | 1 (100%) | |
| Urinary System | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Bilateral, renal tubule, adenoma | | | 1 (2%) | |
| Renal tubule, adenoma | | | 5 (10%) | 3 (6%) |
| Renal tubule, carcinoma | 1 (2%) | | 2 (4%) | |
| Transitional epithelium, papilloma | | | | 1 (2%) |
| Urinary bladder | (49) | (50) | (50) | (50) |
| Transitional epithelium, papilloma | | 3 (6%) | | |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--|----------|----------|----------|----------|
| 2-Year Study (continued) | | | | |
| Systemic Lesions | | | | |
| Multiple organs | (50) | (50) | (50) | (50) |
| Leukemia mononuclear | 34 (68%) | 43 (86%) | 38 (76%) | 31 (62%) |
| Mesothelioma malignant | 5 (10%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | | | | |
| 15-Month interim evaluation | 10 | 9 | 10 | 10 |
| 2-Year study | 49 | 49 | 50 | 49 |
| Total primary neoplasms | | | | |
| 15-Month interim evaluation | 16 | 12 | 13 | 18 |
| 2-Year study | 177 | 175 | 194 | 149 |
| Total animals with benign neoplasms | | | | |
| 15-Month interim evaluation | 10 | 9 | 10 | 10 |
| 2-Year study | 49 | 48 | 50 | 49 |
| Total benign neoplasms | | | | |
| 15-Month interim evaluation | 14 | 12 | 12 | 14 |
| 2-Year study | 126 | 117 | 127 | 106 |
| Total animals with malignant neoplasms | | | | |
| 15-Month interim evaluation | 2 | | 1 | 4 |
| 2-Year study | 39 | 46 | 48 | 35 |
| Total malignant neoplasms | | | | |
| 15-Month interim evaluation | 2 | | 1 | 4 |
| 2-Year study | 51 | 57 | 66 | 43 |
| Total animals with metastatic neoplasms | | | | |
| 2-Year study | 1 | 1 | 1 | 2 |
| Total metastatic neoplasms | | | | |
| 2-Year study | 1 | 1 | 1 | 3 |
| Total animals with malignant neoplasms of uncertain primary site | | | | |
| 2-Year study | | 1 | | 1 |
| Total animals with uncertain neoplasms-benign or malignant | | | | |
| 2-Year study | | 1 | 1 | |
| Total uncertain neoplasms | | | | |
| 2-Year study | | 1 | 1 | |

^a Number of animals examined microscopically at the site and the number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Tetrafluoroethylene: 0 ppm

| | 3 | 3 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | | | |
|------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of Days on Study | 3 | 7 | 8 | 0 | 2 | 5 | 5 | 8 | 9 | 9 | 9 | 2 | 3 | 3 | 4 | 5 | 6 | 7 | 7 | 7 | 8 | 9 | 9 | 0 | 0 | |
| | 6 | 1 | 9 | 9 | 5 | 5 | 9 | 8 | 1 | 3 | 4 | 4 | 2 | 5 | 9 | 9 | 4 | 4 | 7 | 9 | 6 | 0 | 1 | 1 | 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 | 5 | 5 | 1 | 5 | 3 | 5 | 2 | 4 | 5 | 4 | 4 | 0 | 2 | 5 | 0 | 4 | 1 | 2 | 4 | 3 | 0 | 2 | 3 | 3 | |
| | 0 | 2 | 6 | 3 | 0 | 0 | 3 | 8 | 3 | 9 | 8 | 4 | 7 | 3 | 4 | 6 | 7 | 6 | 6 | 1 | 6 | 4 | 7 | 3 | 4 | |
| Alimentary System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine large, colon | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine large, rectum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + |
| Intestine large, cecum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine small, duodenum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine small, jejunum | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine small, ileum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hepatocellular adenoma, multiple | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mesentery | | | | + | | | | | | | | | | | | | | | | | | | | | | |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pharynx | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Palate, squamous cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary glands | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach, forestomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach, glandular | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Tongue | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Tooth | | | | + | | | | | | | | + | | | | | | | | | | | | | | |
| Cardiovascular System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Blood vessel | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Endocrine System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adrenal cortex | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adrenal medulla | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma malignant | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pheochromocytoma benign | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bilateral, pheochromocytoma benign | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Islets, pancreatic | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pituitary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pars distalis, adenoma | X | X | X | | | | X | | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Pars intermedia, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C-cell, carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Follicular cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| General Body System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| None | | | | | | | | | | | | | | | | | | | | | | | | | | |

+ : Tissue examined microscopically
A : Autolysis precludes examination

M : Missing tissue
I : Insufficient tissue

X : Lesion present
Blank : Not examined

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--|----------------|-------------|------------|-------------|
| Adrenal Medulla: Benign Pheochromocytoma | | | | |
| Overall rate ^a | 19/50 (38%) | 11/50 (22%) | 9/50 (18%) | 10/50 (20%) |
| Adjusted rate ^b | 60.0% | 44.2% | 33.4% | 52.8% |
| Terminal rate ^c | 6/17 (35%) | 2/12 (17%) | 4/17 (24%) | 0/1 (0%) |
| First incidence (days) | 588 | 594 | 576 | 481 |
| Life table test ^d | P=0.282 | P=0.280N | P=0.058N | P=0.083 |
| Logistic regression test ^d | P=0.108N | P=0.090N | P=0.024N | P=0.180N |
| Cochran-Armitage test ^d | P=0.040N | | | |
| Fisher exact test ^d | | P=0.063N | P=0.022N | P=0.038N |
| Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma | | | | |
| Overall rate | 20/50 (40%) | 12/50 (24%) | 9/50 (18%) | 10/50 (20%) |
| Adjusted rate | 63.6% | 49.8% | 33.4% | 52.8% |
| Terminal rate | 7/17 (41%) | 3/12 (25%) | 4/17 (24%) | 0/1 (0%) |
| First incidence (days) | 588 | 594 | 576 | 481 |
| Life table test | P=0.331 | P=0.305N | P=0.039N | P=0.087 |
| Logistic regression test | P=0.078N | P=0.123N | P=0.015N | P=0.151N |
| Cochran-Armitage test | P=0.023N | | | |
| Fisher exact test | | P=0.066N | P=0.013N | P=0.024N |
| Kidney (Renal Tubule): Adenoma (Single Sections) | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 6/50 (12%) | 3/50 (6%) |
| Adjusted rate | 0.0% | 0.0% | 25.7% | 18.4% |
| Terminal rate | 0/17 (0%) | 0/12 (0%) | 3/17 (18%) | 0/1 (0%) |
| First incidence (days) | — ^e | — | 627 | 617 |
| Life table test | P<0.001 | — | P=0.015 | P=0.032 |
| Logistic regression test | P=0.015 | — | P=0.015 | P=0.097 |
| Cochran-Armitage test | P=0.047 | | | |
| Fisher exact test | | — | P=0.013 | P=0.121 |
| Kidney (Renal Tubule): Adenoma (Step Sections) | | | | |
| Overall rate | 2/50 (4%) | 4/50 (8%) | 3/50 (6%) | 11/50 (22%) |
| Adjusted rate | 10.8% | 24.9% | 10.2% | 85.3% |
| Terminal rate | 1/17 (6%) | 2/12 (17%) | 0/17 (0%) | 0/1 (0%) |
| First incidence (days) | 721 | 555 | 614 | 572 |
| Life table test | P<0.001 | P=0.217 | P=0.506 | P<0.001 |
| Logistic regression test | P<0.001 | P=0.259 | P=0.492 | P<0.001 |
| Cochran-Armitage test | P=0.002 | | | |
| Fisher exact test | | P=0.339 | P=0.500 | P=0.007 |
| Kidney (Renal Tubule): Adenoma (Single and Step Sections) | | | | |
| Overall rate | 2/50 (4%) | 4/50 (8%) | 9/50 (18%) | 13/50 (26%) |
| Adjusted rate | 10.8% | 24.9% | 33.3% | 87.7% |
| Terminal rate | 1/17 (6%) | 2/12 (17%) | 3/17 (18%) | 0/1 (0%) |
| First incidence (days) | 721 | 555 | 614 | 572 |
| Life table test | P<0.001 | P=0.217 | P=0.029 | P<0.001 |
| Logistic regression test | P<0.001 | P=0.259 | P=0.024 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P=0.339 | P=0.026 | P=0.002 |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|---|------------|------------|-------------|-------------|
| Kidney (Renal Tubule): Adenoma or Carcinoma (Single Sections) | | | | |
| Overall rate | 1/50 (2%) | 0/50 (0%) | 6/50 (12%) | 3/50 (6%) |
| Adjusted rate | 2.8% | 0.0% | 25.7% | 18.4% |
| Terminal rate | 0/17 (0%) | 0/12 (0%) | 3/17 (18%) | 0/1 (0%) |
| First incidence (days) | 649 | — | 627 | 617 |
| Life table test | P=0.004 | P=0.543N | P=0.049 | P=0.094 |
| Logistic regression test | P=0.052 | P=0.497N | P=0.055 | P=0.270 |
| Cochran-Armitage test | P=0.114 | | | |
| Fisher exact test | | P=0.500N | P=0.056 | P=0.309 |
| Kidney (Renal Tubule): Adenoma or Carcinoma (Step Sections) | | | | |
| Overall rate | 2/50 (4%) | 5/50 (10%) | 3/50 (6%) | 11/50 (22%) |
| Adjusted rate | 10.8% | 28.4% | 10.2% | 85.3% |
| Terminal rate | 1/17 (6%) | 2/12 (17%) | 0/17 (0%) | 0/1 (0%) |
| First incidence (days) | 721 | 555 | 614 | 572 |
| Life table test | P<0.001 | P=0.123 | P=0.506 | P=0.001 |
| Logistic regression test | P<0.001 | P=0.151 | P=0.492 | P<0.001 |
| Cochran-Armitage test | P=0.004 | | | |
| Fisher exact test | | P=0.218 | P=0.500 | P=0.007 |
| Kidney (Renal Tubule): Adenoma or Carcinoma (Single and Step Sections) | | | | |
| Overall rate | 3/50 (6%) | 5/50 (10%) | 9/50 (18%) | 13/50 (26%) |
| Adjusted rate | 13.3% | 28.4% | 33.3% | 87.7% |
| Terminal rate | 1/17 (6%) | 2/12 (17%) | 3/17 (18%) | 0/1 (0%) |
| First incidence (days) | 649 | 555 | 614 | 572 |
| Life table test | P<0.001 | P=0.224 | P=0.060 | P<0.001 |
| Logistic regression test | P<0.001 | P=0.281 | P=0.057 | P<0.001 |
| Cochran-Armitage test | P=0.002 | | | |
| Fisher exact test | | P=0.357 | P=0.061 | P=0.006 |
| Liver: Hepatocellular Adenoma | | | | |
| Overall rate | 3/50 (6%) | 6/50 (12%) | 8/50 (16%) | 5/50 (10%) |
| Adjusted rate | 14.1% | 32.0% | 43.1% | 100.0% |
| Terminal rate | 2/17 (12%) | 3/12 (25%) | 7/17 (41%) | 1/1 (100%) |
| First incidence (days) | 632 | 600 | 638 | 572 |
| Life table test | P=0.004 | P=0.127 | P=0.082 | P=0.018 |
| Logistic regression test | P=0.103 | P=0.193 | P=0.065 | P=0.240 |
| Cochran-Armitage test | P=0.352 | | | |
| Fisher exact test | | P=0.243 | P=0.100 | P=0.357 |
| Liver: Hepatocellular Carcinoma | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 10/50 (20%) | 3/50 (6%) |
| Adjusted rate | 2.6% | 3.4% | 41.3% | 18.8% |
| Terminal rate | 0/17 (0%) | 0/12 (0%) | 5/17 (29%) | 0/1 (0%) |
| First incidence (days) | 624 | 652 | 617 | 590 |
| Life table test | P=0.003 | P=0.710 | P=0.005 | P=0.113 |
| Logistic regression test | P=0.053 | P=0.756N | P=0.004 | P=0.301 |
| Cochran-Armitage test | P=0.149 | | | |
| Fisher exact test | | P=0.753N | P=0.004 | P=0.309 |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|---|------------|------------|-------------|------------|
| Liver: Hepatocellular Adenoma or Carcinoma | | | | |
| Overall rate | 4/50 (8%) | 7/50 (14%) | 15/50 (30%) | 8/50 (16%) |
| Adjusted rate | 16.3% | 34.4% | 62.1% | 100.0% |
| Terminal rate | 2/17 (12%) | 3/12 (25%) | 9/17 (53%) | 1/1 (100%) |
| First incidence (days) | 624 | 600 | 617 | 572 |
| Life table test | P<0.001 | P=0.137 | P=0.004 | P=0.003 |
| Logistic regression test | P=0.025 | P=0.223 | P=0.003 | P=0.112 |
| Cochran-Armitage test | P=0.140 | | | |
| Fisher exact test | | P=0.262 | P=0.005 | P=0.178 |
| Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma | | | | |
| Overall rate | 2/50 (4%) | 2/50 (4%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate | 10.4% | 12.7% | 14.2% | 11.1% |
| Terminal rate | 1/17 (6%) | 1/12 (8%) | 2/17 (12%) | 0/1 (0%) |
| First incidence (days) | 715 | 698 | 627 | 523 |
| Life table test | P=0.117 | P=0.580 | P=0.491 | P=0.229 |
| Logistic regression test | P=0.386 | P=0.598 | P=0.463 | P=0.631 |
| Cochran-Armitage test | P=0.569 | | | |
| Fisher exact test | | P=0.691N | P=0.500 | P=0.691N |
| Pancreatic Islets: Adenoma | | | | |
| Overall rate | 3/50 (6%) | 1/50 (2%) | 1/50 (2%) | 2/50 (4%) |
| Adjusted rate | 15.3% | 5.6% | 5.9% | 20.5% |
| Terminal rate | 2/17 (12%) | 0/12 (0%) | 1/17 (6%) | 0/1 (0%) |
| First incidence (days) | 705 | 705 | 733 (T) | 626 |
| Life table test | P=0.248 | P=0.433N | P=0.324N | P=0.182 |
| Logistic regression test | P=0.463 | P=0.400N | P=0.339N | P=0.521 |
| Cochran-Armitage test | P=0.475N | | | |
| Fisher exact test | | P=0.309N | P=0.309N | P=0.500N |
| Pancreatic Islets: Carcinoma | | | | |
| Overall rate | 1/50 (2%) | 5/50 (10%) | 1/50 (2%) | 1/50 (2%) |
| Adjusted rate | 3.4% | 31.5% | 5.9% | 3.0% |
| Terminal rate | 0/17 (0%) | 3/12 (25%) | 1/17 (6%) | 0/1 (0%) |
| First incidence (days) | 690 | 664 | 733 (T) | 593 |
| Life table test | P=0.432 | P=0.049 | P=0.730 | P=0.587 |
| Logistic regression test | P=0.550N | P=0.059 | P=0.744 | P=0.768 |
| Cochran-Armitage test | P=0.313N | | | |
| Fisher exact test | | P=0.102 | P=0.753N | P=0.753N |
| Pancreatic Islets: Adenoma or Carcinoma | | | | |
| Overall rate | 4/50 (8%) | 6/50 (12%) | 2/50 (4%) | 3/50 (6%) |
| Adjusted rate | 18.2% | 35.3% | 11.8% | 22.9% |
| Terminal rate | 2/17 (12%) | 3/12 (25%) | 2/17 (12%) | 0/1 (0%) |
| First incidence (days) | 690 | 664 | 733 (T) | 593 |
| Life table test | P=0.202 | P=0.192 | P=0.375N | P=0.144 |
| Logistic regression test | P=0.525 | P=0.228 | P=0.387N | P=0.553 |
| Cochran-Armitage test | P=0.278N | | | |
| Fisher exact test | | P=0.370 | P=0.339N | P=0.500N |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|---|-------------|-------------|-------------|-------------|
| Pituitary Gland (Pars Distalis): Adenoma | | | | |
| Overall rate | 39/50 (78%) | 37/49 (76%) | 35/49 (71%) | 23/49 (47%) |
| Adjusted rate | 94.9% | 91.1% | 84.5% | 86.1% |
| Terminal rate | 15/17 (88%) | 9/12 (75%) | 10/16 (63%) | 0/0 |
| First incidence (days) | 336 | 351 | 559 | 361 |
| Life table test | P=0.042 | P=0.213 | P=0.514N | P=0.003 |
| Logistic regression test | P=0.002N | P=0.500N | P=0.290N | P=0.014N |
| Cochran-Armitage test | P<0.001N | | | |
| Fisher exact test | | P=0.478N | P=0.301N | P=0.001N |
| Preputial Gland: Adenoma | | | | |
| Overall rate | 1/50 (2%) | 3/50 (6%) | 5/50 (10%) | 2/49 (4%) |
| Adjusted rate | 5.9% | 25.0% | 15.6% | 6.0% |
| Terminal rate | 1/17 (6%) | 3/12 (25%) | 1/17 (6%) | 0/1 (0%) |
| First incidence (days) | 733 (T) | 733 (T) | 538 | 523 |
| Life table test | P=0.099 | P=0.182 | P=0.104 | P=0.228 |
| Logistic regression test | P=0.393 | P=0.182 | P=0.099 | P=0.523 |
| Cochran-Armitage test | P=0.428 | | | |
| Fisher exact test | | P=0.309 | P=0.102 | P=0.492 |
| Preputial Gland: Carcinoma | | | | |
| Overall rate | 3/50 (6%) | 3/50 (6%) | 2/50 (4%) | 4/49 (8%) |
| Adjusted rate | 15.6% | 10.0% | 4.9% | 13.9% |
| Terminal rate | 2/17 (12%) | 0/12 (0%) | 0/17 (0%) | 0/1 (0%) |
| First incidence (days) | 708 | 494 | 538 | 481 |
| Life table test | P=0.152 | P=0.552 | P=0.515N | P=0.077 |
| Logistic regression test | P=0.481 | P=0.656 | P=0.500N | P=0.471 |
| Cochran-Armitage test | P=0.409 | | | |
| Fisher exact test | | P=0.661N | P=0.500N | P=0.489 |
| Preputial Gland: Adenoma or Carcinoma | | | | |
| Overall rate | 4/50 (8%) | 6/50 (12%) | 6/50 (12%) | 6/49 (12%) |
| Adjusted rate | 21.2% | 32.5% | 18.1% | 19.0% |
| Terminal rate | 3/17 (18%) | 3/12 (25%) | 1/17 (6%) | 0/1 (0%) |
| First incidence (days) | 708 | 494 | 538 | 481 |
| Life table test | P=0.035 | P=0.211 | P=0.350 | P=0.023 |
| Logistic regression test | P=0.355 | P=0.295 | P=0.369 | P=0.354 |
| Cochran-Armitage test | P=0.337 | | | |
| Fisher exact test | | P=0.370 | P=0.370 | P=0.357 |
| Skin: Keratoacanthoma | | | | |
| Overall rate | 3/50 (6%) | 0/50 (0%) | 1/50 (2%) | 1/50 (2%) |
| Adjusted rate | 14.7% | 0.0% | 2.2% | 5.3% |
| Terminal rate | 2/17 (12%) | 0/12 (0%) | 0/17 (0%) | 0/1 (0%) |
| First incidence (days) | 686 | — | 575 | 632 |
| Life table test | P=0.579N | P=0.184N | P=0.326N | P=0.598 |
| Logistic regression test | P=0.356N | P=0.165N | P=0.309N | P=0.609N |
| Cochran-Armitage test | P=0.296N | | | |
| Fisher exact test | | P=0.121N | P=0.309N | P=0.309N |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--|-------------|-------------|--------------|-------------|
| Skin: Squamous Cell Papilloma or Keratoacanthoma | | | | |
| Overall rate | 4/50 (8%) | 0/50 (0%) | 1/50 (2%) | 2/50 (4%) |
| Adjusted rate | 19.0% | 0.0% | 2.2% | 8.9% |
| Terminal rate | 2/17 (12%) | 0/12 (0%) | 0/17 (0%) | 0/1 (0%) |
| First incidence (days) | 686 | — | 575 | 617 |
| Life table test | P=0.525 | P=0.112N | P=0.197N | P=0.375 |
| Logistic regression test | P=0.452N | P=0.095N | P=0.186N | P=0.629N |
| Cochran-Armitage test | P=0.373N | | | |
| Fisher exact test | | P=0.059N | P=0.181N | P=0.339N |
| Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma | | | | |
| Overall rate | 5/50 (10%) | 0/50 (0%) | 2/50 (4%) | 2/50 (4%) |
| Adjusted rate | 23.5% | 0.0% | 7.7% | 8.9% |
| Terminal rate | 2/17 (12%) | 0/12 (0%) | 0/17 (0%) | 0/1 (0%) |
| First incidence (days) | 686 | — | 575 | 617 |
| Life table test | P=0.552 | P=0.071N | P=0.233N | P=0.421 |
| Logistic regression test | P=0.401N | P=0.056N | P=0.236N | P=0.550N |
| Cochran-Armitage test | P=0.263N | | | |
| Fisher exact test | | P=0.028N | P=0.218N | P=0.218N |
| Skin: Squamous Cell Papilloma, Keratoacanthoma, Benign Basosquamous Tumor, or Squamous Cell Carcinoma | | | | |
| Overall rate | 7/50 (14%) | 0/50 (0%) | 2/50 (4%) | 3/50 (6%) |
| Adjusted rate | 32.3% | 0.0% | 7.7% | 12.6% |
| Terminal rate | 3/17 (18%) | 0/12 (0%) | 0/17 (0%) | 0/1 (0%) |
| First incidence (days) | 686 | — | 575 | 617 |
| Life table test | P=0.527 | P=0.029N | P=0.092N | P=0.301 |
| Logistic regression test | P=0.369N | P=0.019N | P=0.095N | P=0.529N |
| Cochran-Armitage test | P=0.211N | | | |
| Fisher exact test | | P=0.006N | P=0.080N | P=0.159N |
| Skin (Subcutaneous Tissue): Fibroma | | | | |
| Overall rate | 3/50 (6%) | 1/50 (2%) | 0/50 (0%) | 1/50 (2%) |
| Adjusted rate | 11.3% | 5.6% | 0.0% | 2.6% |
| Terminal rate | 1/17 (6%) | 0/12 (0%) | 0/17 (0%) | 0/1 (0%) |
| First incidence (days) | 635 | 705 | — | 571 |
| Life table test | P=0.430N | P=0.420N | P=0.150N | P=0.700N |
| Logistic regression test | P=0.224N | P=0.343N | P=0.122N | P=0.346N |
| Cochran-Armitage test | P=0.200N | | | |
| Fisher exact test | | P=0.309N | P=0.121N | P=0.309N |
| Testes: Adenoma | | | | |
| Overall rate | 39/50 (78%) | 40/50 (80%) | 48/50 (96%) | 47/50 (94%) |
| Adjusted rate | 92.5% | 97.3% | 100.0% | 100.0% |
| Terminal rate | 14/17 (82%) | 11/12 (92%) | 17/17 (100%) | 1/1 (100%) |
| First incidence (days) | 489 | 470 | 413 | 418 |
| Life table test | P<0.001 | P=0.101 | P=0.081 | P<0.001 |
| Logistic regression test | P<0.001 | P=0.361 | P=0.009 | P=0.003 |
| Cochran-Armitage test | P=0.004 | | | |
| Fisher exact test | | P=0.500 | P=0.007 | P=0.020 |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|---|-------------|-------------|-------------|-------------|
| Thyroid Gland (C-cell): Adenoma | | | | |
| Overall rate | 7/50 (14%) | 8/50 (16%) | 5/50 (10%) | 4/50 (8%) |
| Adjusted rate | 31.8% | 37.3% | 21.6% | 20.6% |
| Terminal rate | 3/17 (18%) | 3/12 (25%) | 2/17 (12%) | 0/1 (0%) |
| First incidence (days) | 707 | 533 | 631 | 571 |
| Life table test | P=0.283 | P=0.286 | P=0.437N | P=0.128 |
| Logistic regression test | P=0.348N | P=0.386 | P=0.438N | P=0.636 |
| Cochran-Armitage test | P=0.152N | | | |
| Fisher exact test | | P=0.500 | P=0.380N | P=0.262N |
| Thyroid Gland (C-cell): Carcinoma | | | | |
| Overall rate | 2/50 (4%) | 1/50 (2%) | 4/50 (8%) | 1/50 (2%) |
| Adjusted rate | 11.8% | 8.3% | 18.4% | 3.8% |
| Terminal rate | 2/17 (12%) | 1/12 (8%) | 2/17 (12%) | 0/1 (0%) |
| First incidence (days) | 733 (T) | 733 (T) | 658 | 617 |
| Life table test | P=0.204 | P=0.623N | P=0.307 | P=0.508 |
| Logistic regression test | P=0.403 | P=0.623N | P=0.291 | P=0.754 |
| Cochran-Armitage test | P=0.500N | | | |
| Fisher exact test | | P=0.500N | P=0.339 | P=0.500N |
| Thyroid Gland (C-cell): Adenoma or Carcinoma | | | | |
| Overall rate | 8/50 (16%) | 9/50 (18%) | 9/50 (18%) | 5/50 (10%) |
| Adjusted rate | 36.7% | 44.3% | 37.2% | 23.7% |
| Terminal rate | 4/17 (24%) | 4/12 (33%) | 4/17 (24%) | 0/1 (0%) |
| First incidence (days) | 707 | 533 | 631 | 571 |
| Life table test | P=0.105 | P=0.263 | P=0.433 | P=0.073 |
| Logistic regression test | P=0.530N | P=0.357 | P=0.420 | P=0.577 |
| Cochran-Armitage test | P=0.204N | | | |
| Fisher exact test | | P=0.500 | P=0.500 | P=0.277N |
| Urinary Bladder: Papilloma | | | | |
| Overall rate | 0/49 (0%) | 3/50 (6%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate | 0.0% | 14.8% | 0.0% | 0.0% |
| Terminal rate | 0/17 (0%) | 1/12 (8%) | 0/17 (0%) | 0/1 (0%) |
| First incidence (days) | — | 600 | — | — |
| Life table test | P=0.509N | P=0.085 | — | — |
| Logistic regression test | P=0.354N | P=0.113 | — | — |
| Cochran-Armitage test | P=0.308N | | | |
| Fisher exact test | | P=0.125 | — | — |
| All Organs: Mononuclear Cell Leukemia | | | | |
| Overall rate | 34/50 (68%) | 43/50 (86%) | 38/50 (76%) | 31/50 (62%) |
| Adjusted rate | 81.5% | 95.3% | 91.9% | 100.0% |
| Terminal rate | 10/17 (59%) | 10/12 (83%) | 14/17 (82%) | 1/1 (100%) |
| First incidence (days) | 509 | 351 | 413 | 509 |
| Life table test | P<0.001 | P=0.016 | P=0.231 | P<0.001 |
| Logistic regression test | P=0.064N | P=0.020 | P=0.254 | P=0.079N |
| Cochran-Armitage test | P=0.108N | | | |
| Fisher exact test | | P=0.028 | P=0.252 | P=0.338N |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--|--------------|--------------|--------------|-------------|
| All Organs: Malignant Mesothelioma | | | | |
| Overall rate | 5/50 (10%) | 1/50 (2%) | 2/50 (4%) | 1/50 (2%) |
| Adjusted rate | 15.0% | 3.8% | 9.5% | 2.3% |
| Terminal rate | 1/17 (6%) | 0/12 (0%) | 1/17 (6%) | 0/1 (0%) |
| First incidence (days) | 489 | 664 | 674 | 525 |
| Life table test | P=0.248N | P=0.154N | P=0.253N | P=0.278N |
| Logistic regression test | P=0.076N | P=0.089N | P=0.222N | P=0.062N |
| Cochran-Armitage test | P=0.093N | | | |
| Fisher exact test | | P=0.102N | P=0.218N | P=0.102N |
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 49/50 (98%) | 48/50 (96%) | 50/50 (100%) | 49/50 (98%) |
| Adjusted rate | 100.0% | 100.0% | 100.0% | 100.0% |
| Terminal rate | 17/17 (100%) | 12/12 (100%) | 17/17 (100%) | 1/1 (100%) |
| First incidence (days) | 336 | 351 | 413 | 361 |
| Life table test | P<0.001 | P=0.130 | P=0.365 | P=0.001 |
| Logistic regression test | P=0.460 | P=0.529N | P=0.894 | P=0.759N |
| Cochran-Armitage test | P=0.500 | | | |
| Fisher exact test | | P=0.500N | P=0.500 | P=0.753N |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 39/50 (78%) | 46/50 (92%) | 48/50 (96%) | 35/50 (70%) |
| Adjusted rate | 87.9% | 97.8% | 98.0% | 100.0% |
| Terminal rate | 12/17 (71%) | 11/12 (92%) | 16/17 (94%) | 1/1 (100%) |
| First incidence (days) | 489 | 351 | 413 | 481 |
| Life table test | P<0.001 | P=0.025 | P=0.087 | P<0.001 |
| Logistic regression test | P=0.035N | P=0.030 | P=0.009 | P=0.042N |
| Cochran-Armitage test | P=0.075N | | | |
| Fisher exact test | | P=0.045 | P=0.007 | P=0.247N |
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 49/50 (98%) | 49/50 (98%) | 50/50 (100%) | 49/50 (98%) |
| Adjusted rate | 100.0% | 100.0% | 100.0% | 100.0% |
| Terminal rate | 17/17 (100%) | 12/12 (100%) | 17/17 (100%) | 1/1 (100%) |
| First incidence (days) | 336 | 351 | 413 | 361 |
| Life table test | P<0.001 | P=0.105 | P=0.365 | P<0.001 |
| Logistic regression test | P=0.655N | P=0.769N | P=0.894 | P=0.759N |
| Cochran-Armitage test | P=0.617 | | | |
| Fisher exact test | | P=0.753N | P=0.500 | 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, liver, pancreatic islets, pituitary gland, preputial gland, testes, thyroid gland, and urinary bladder; 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 A4a
Historical Incidence of Renal Tubule Neoplasms in Chamber Control Male F344/N Rats^a

| Study | Incidence in Controls | | |
|--|-----------------------|------------|----------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence at Battelle Pacific Northwest Laboratories | | | |
| <i>o</i> -Chlorobenzalmononitrile (CS-2) | 1/50 | 0/50 | 1/50 |
| Acetonitrile | 1/48 | 0/48 | 1/48 |
| 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 | 0/50 | 0/50 | 0/50 |
| Ozone | 2/50 | 0/50 | 2/50 |
| Total | 5/347 (1.4%) | 0/347 (0%) | 5/347 (1.4%) |
| Standard deviation | 1.5% | | 1.5% |
| Range | 0%-4% | | 0%-4% |
| Overall Historical Incidence | | | |
| Total | 6/652 (0.9%) | 0/652 (0%) | 6/652 (0.9%) |
| Standard deviation | 1.3% | | 1.3% |
| Range | 0%-4% | | 0%-4% |

^a Data as of 12 May 1995

TABLE A4b
Historical Incidence of Hepatocellular Neoplasms in Chamber Control Male F344/N Rats^a

| Study | Incidence in Controls | | |
|--|-----------------------|--------------|----------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence at Battelle Pacific Northwest Laboratories | | | |
| <i>o</i> -Chlorobenzalmononitrile (CS-2) | 2/50 | 2/50 | 4/50 |
| Acetonitrile | 0/48 | 1/48 | 1/48 |
| α -Chloroacetophenone | 1/49 | 0/49 | 1/49 |
| Epinephrine Hydrochloride | 1/50 | 0/50 | 1/50 |
| Ethyl Chloride | 0/50 | 1/50 | 1/50 |
| Hexachlorocyclopentadiene | 1/50 | 0/50 | 1/50 |
| Ozone | 2/50 | 0/50 | 2/50 |
| Total | 7/347 (2.0%) | 4/347 (1.2%) | 11/347 (3.2%) |
| Standard deviation | 1.6% | 1.6% | 2.3% |
| Range | 0%-4% | 0%-4% | 2%-8% |
| Overall Historical Incidence | | | |
| Total | 20/653 (3.1%) | 8/653 (1.2%) | 28/653 (4.3%) |
| Standard deviation | 2.8% | 1.5% | 2.9% |
| Range | 0%-8% | 0%-4% | 2%-9% |

^a Data as of 12 May 1995

TABLE A4c
Historical Incidence of Mononuclear Cell Leukemia in Chamber Control Male F344/N Rats ^a

| Study | Incidence in Controls |
|--|-----------------------|
| Historical Incidence at Battelle Pacific Northwest Laboratories | |
| <i>o</i> -Chlorobenzalmalononitrile (CS-2) | 29/50 |
| Acetonitrile | 29/48 |
| α -Chloroacetophenone | 29/50 |
| Epinephrine Hydrochloride | 19/50 |
| Ethyl Chloride | 33/50 |
| Hexachlorocyclopentadiene | 29/50 |
| Ozone | 27/50 |
| Total | 195/348 (56.0%) |
| Standard deviation | 8.7% |
| Range | 38%-66% |
| Overall Historical Incidence | |
| Total | 356/655 (54.4%) |
| Standard deviation | 8.8% |
| Range | 34%-66% |

^a Data as of 12 May 1995; includes data for lymphocytic, monocytic, and undifferentiated cell type leukemias

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study
of Tetrafluoroethylene^a

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|------------------------------------|-----------|-----------|-----------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation | | | | |
| Early deaths | | | | |
| Moribund | 27 | 34 | 32 | 48 |
| Natural deaths | 6 | 4 | 1 | 1 |
| Survivors | | | | |
| Terminal sacrifice | 17 | 12 | 17 | 1 |
| Animals examined microscopically | 60 | 60 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Intestine large, cecum | (10) | (10) | (10) | (10) |
| Fibrosis | | | | 1 (10%) |
| Liver | (10) | (10) | (10) | (10) |
| Basophilic focus | 6 (60%) | 4 (40%) | 7 (70%) | 4 (40%) |
| Clear cell focus | 4 (40%) | 10 (100%) | 9 (90%) | 10 (100%) |
| Degeneration, cystic | | 1 (10%) | 3 (30%) | |
| Eosinophilic focus | 1 (10%) | | | 1 (10%) |
| Hepatodiaphragmatic nodule | 1 (10%) | | 1 (10%) | 1 (10%) |
| Mixed cell focus | | 2 (20%) | 2 (20%) | 2 (20%) |
| Regeneration | | | | 1 (10%) |
| Bile duct, hyperplasia | 7 (70%) | 9 (90%) | 6 (60%) | 3 (30%) |
| Serosa, fibrosis | | | 1 (10%) | |
| Mesentery | (2) | | (2) | (4) |
| Fat, hemorrhage | 1 (50%) | | | |
| Fat, necrosis | 1 (50%) | | 2 (100%) | 3 (75%) |
| Pancreas | (10) | (10) | (10) | (10) |
| Basophilic focus | | | 2 (20%) | |
| Hyperplasia | | | 1 (10%) | |
| Acinus, atrophy | 4 (40%) | 7 (70%) | 2 (20%) | 4 (40%) |
| Stomach, glandular | (10) | (10) | (10) | (10) |
| Mineralization | 1 (10%) | | | |
| Cardiovascular System | | | | |
| Heart | (10) | (10) | (10) | (10) |
| Cardiomyopathy | 10 (100%) | 10 (100%) | 10 (100%) | 10 (100%) |
| Endocrine System | | | | |
| Adrenal cortex | (10) | (10) | (10) | (10) |
| Hyperplasia | 1 (10%) | 2 (20%) | 1 (10%) | 2 (20%) |
| Adrenal medulla | (10) | (10) | (10) | (10) |
| Hyperplasia | 3 (30%) | | | 1 (10%) |
| Pituitary gland | (10) | (9) | (10) | (10) |
| Pars distalis, hyperplasia | 6 (60%) | 3 (33%) | 4 (40%) | 7 (70%) |
| Thyroid gland | (10) | (10) | (10) | (10) |
| C-cell, hyperplasia | 4 (40%) | 6 (60%) | 5 (50%) | 6 (60%) |

^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 Tetrafluoroethylene (continued)

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--|-----------|-----------|-----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Genital System | | | | |
| Preputial gland | (10) | (10) | (10) | (10) |
| Inflammation, chronic active | 3 (30%) | 8 (80%) | 7 (70%) | 6 (60%) |
| Testes | (10) | (10) | (10) | (10) |
| Atrophy | | 1 (10%) | | |
| Interstitial cell, hyperplasia | 2 (20%) | 4 (40%) | | 1 (10%) |
| Hematopoietic System | | | | |
| Bone marrow | (10) | (10) | (10) | (10) |
| Hyperplasia, reticulum cell | 1 (10%) | | 1 (10%) | |
| Lymph node, mandibular | (10) | (8) | (10) | (10) |
| Hemorrhage | 1 (10%) | | | |
| Lymph node, mesenteric | (10) | (10) | (10) | (10) |
| Inflammation, chronic active | 1 (10%) | | | |
| Lymph node, mediastinal | (10) | (10) | (10) | (10) |
| Hemorrhage | 2 (20%) | 1 (10%) | 2 (20%) | 1 (10%) |
| Spleen | (10) | (10) | (10) | (10) |
| Developmental malformation | 2 (20%) | | 1 (10%) | |
| Capsule, fibrosis | | | 1 (10%) | |
| Integumentary System | | | | |
| Mammary gland | (5) | (7) | (4) | (5) |
| Galactocele | | | | 1 (20%) |
| Skin | (10) | (10) | (10) | (10) |
| Inflammation, chronic active | | 1 (10%) | 1 (10%) | |
| Respiratory System | | | | |
| Larynx | (10) | (10) | (10) | (10) |
| Inflammation, chronic active | 4 (40%) | 5 (50%) | 5 (50%) | 7 (70%) |
| Metaplasia, squamous | | | 1 (10%) | |
| Mineralization | 6 (60%) | 8 (80%) | 8 (80%) | 9 (90%) |
| Lung | (10) | (10) | (10) | (10) |
| Hemorrhage | 10 (100%) | 10 (100%) | 10 (100%) | 10 (100%) |
| Metaplasia, osseous | | | | 1 (10%) |
| Alveolar epithelium, hyperplasia | 1 (10%) | 2 (20%) | 3 (30%) | |
| Urinary System | | | | |
| Kidney | (10) | (10) | (10) | (10) |
| Cyst | | 1 (10%) | | |
| Nephropathy | 10 (100%) | 10 (100%) | 10 (100%) | 10 (100%) |
| Renal tubule, degeneration | 1 (10%) | 8 (80%) | 10 (100%) | 10 (100%) |
| Renal tubule, hyperplasia | | | 1 (10%) | 1 (10%) |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--|----------|-----------|----------|----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Systems Examined With No Lesions Observed | | | | |
| General Body System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |
| Special Senses System | | | | |
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Intestine large, colon | (50) | (50) | (50) | (50) |
| Mineralization | 1 (2%) | | 2 (4%) | 1 (2%) |
| Parasite metazoan | 2 (4%) | 1 (2%) | | 3 (6%) |
| Intestine large, rectum | (49) | (50) | (50) | (50) |
| Parasite metazoan | 3 (6%) | 1 (2%) | | 2 (4%) |
| Intestine large, cecum | (50) | (50) | (50) | (50) |
| Inflammation, suppurative | | 1 (2%) | | |
| Parasite metazoan | | 2 (4%) | 1 (2%) | |
| Intestine small, duodenum | (50) | (50) | (50) | (50) |
| Necrosis | 1 (2%) | | | |
| Intestine small, ileum | (49) | (49) | (50) | (50) |
| Inflammation, chronic active | | 1 (2%) | | 1 (2%) |
| Mineralization | | | | 1 (2%) |
| Peyer's patch, inflammation, granulomatous | | | | 1 (2%) |
| Liver | (50) | (50) | (50) | (50) |
| Angiectasis | | 3 (6%) | 2 (4%) | 3 (6%) |
| Basophilic focus | 22 (44%) | 19 (38%) | 33 (66%) | 29 (58%) |
| Clear cell focus | 7 (14%) | 8 (16%) | 11 (22%) | 3 (6%) |
| Congestion | | | | 1 (2%) |
| Cyst | | | | 1 (2%) |
| Degeneration, cystic | 17 (34%) | 39 (78%) | 35 (70%) | 32 (64%) |
| Eosinophilic focus | 3 (6%) | 18 (36%) | 22 (44%) | 19 (38%) |
| Fatty change | 5 (10%) | 3 (6%) | 4 (8%) | 5 (10%) |
| Fibrosis | | | | 1 (2%) |
| Hepatodiaphragmatic nodule | 2 (4%) | 2 (4%) | 3 (6%) | 4 (8%) |
| Hyperplasia | | | | 1 (2%) |
| Mineralization | | | | 1 (2%) |
| Mixed cell focus | 5 (10%) | 5 (10%) | 16 (32%) | 13 (26%) |
| Necrosis, focal | | | 1 (2%) | |
| Regeneration | 3 (6%) | 4 (8%) | 1 (2%) | |
| Thrombosis | | | 1 (2%) | |
| Bile duct, hyperplasia | 44 (88%) | 43 (86%) | 44 (88%) | 42 (84%) |
| Centrilobular, necrosis | 3 (6%) | 1 (2%) | 1 (2%) | |
| Mesentery | (15) | (11) | (18) | (14) |
| Inflammation, suppurative | 2 (13%) | | | |
| Fat, hemorrhage | 1 (7%) | | 3 (17%) | 1 (7%) |
| Fat, necrosis | 11 (73%) | 11 (100%) | 16 (89%) | 12 (86%) |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--------------------------------------|----------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Alimentary System (continued) | | | | |
| Pancreas | (50) | (50) | (50) | (50) |
| Basophilic focus | | 4 (8%) | 1 (2%) | 4 (8%) |
| Hyperplasia | 3 (6%) | 1 (2%) | 3 (6%) | |
| Acinus, atrophy | 32 (64%) | 25 (50%) | 31 (62%) | 20 (40%) |
| Duct, concretion | 1 (2%) | | | |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Acanthosis | 2 (4%) | 1 (2%) | 2 (4%) | 5 (10%) |
| Diverticulum | 2 (4%) | 1 (2%) | 2 (4%) | |
| Infiltration cellular, mast cell | 1 (2%) | | 1 (2%) | |
| Mineralization | 1 (2%) | | 2 (4%) | 3 (6%) |
| Necrosis | 6 (12%) | 4 (8%) | 1 (2%) | 6 (12%) |
| Stomach, glandular | (50) | (50) | (50) | (50) |
| Mineralization | 1 (2%) | 3 (6%) | 4 (8%) | 13 (26%) |
| Necrosis | 5 (10%) | 3 (6%) | | 3 (6%) |
| Tongue | (1) | (1) | | (1) |
| Epithelium, hyperplasia | | | | 1 (100%) |
| Tooth | (2) | (2) | (1) | (2) |
| Dysplasia | 2 (100%) | 2 (100%) | 1 (100%) | 2 (100%) |
| Cardiovascular System | | | | |
| Blood vessel | (1) | | (3) | (12) |
| Mineralization | 1 (100%) | | 2 (67%) | 3 (25%) |
| Aorta, mineralization | 1 (100%) | | 3 (100%) | 12 (100%) |
| Heart | (50) | (50) | (50) | (50) |
| Cardiomyopathy | 46 (92%) | 44 (88%) | 43 (86%) | 48 (96%) |
| Mineralization | 1 (2%) | | 2 (4%) | 4 (8%) |
| Atrium, thrombosis | 2 (4%) | 3 (6%) | 1 (2%) | 1 (2%) |
| Endocrine System | | | | |
| Adrenal cortex | (50) | (50) | (50) | (50) |
| Hyperplasia | 16 (32%) | 13 (26%) | 21 (42%) | 17 (34%) |
| Hypertrophy | 4 (8%) | 4 (8%) | 5 (10%) | 4 (8%) |
| Vacuolization cytoplasmic | 2 (4%) | | | |
| Adrenal medulla | (50) | (50) | (50) | (50) |
| Hyperplasia | 21 (42%) | 21 (42%) | 23 (46%) | 26 (52%) |
| Islets, pancreatic | (50) | (50) | (50) | (50) |
| Hyperplasia | 1 (2%) | | | 1 (2%) |
| Parathyroid gland | (50) | (50) | (50) | (49) |
| Hyperplasia | 1 (2%) | 3 (6%) | 7 (14%) | 25 (51%) |
| Pituitary gland | (50) | (49) | (49) | (49) |
| Thrombosis | | 1 (2%) | | |
| Pars distalis, angiectasis | | | 1 (2%) | |
| Pars distalis, hyperplasia | 4 (8%) | 3 (6%) | 4 (8%) | 8 (16%) |
| Thyroid gland | (50) | (50) | (50) | (50) |
| C-cell, hyperplasia | 22 (44%) | 24 (48%) | 35 (70%) | 20 (40%) |
| Follicular cell, hyperplasia | 2 (4%) | | | 2 (4%) |
| General Body System | | | | |
| None | | | | |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|------------------------------------|----------|----------|----------|----------|
| 2-Year Study (continued) | | | | |
| Genital System | | | | |
| Epididymis | (50) | (50) | (50) | (50) |
| Granuloma sperm | | | 1 (2%) | |
| Thrombosis | | 1 (2%) | | |
| Preputial gland | (50) | (50) | (50) | (49) |
| Hyperplasia | | 1 (2%) | 1 (2%) | |
| Inflammation, chronic active | 10 (20%) | 8 (16%) | 10 (20%) | 14 (29%) |
| Inflammation, granulomatous | 1 (2%) | | | |
| Duct, ectasia | 1 (2%) | | 1 (2%) | |
| Prostate | (50) | (50) | (50) | (50) |
| Inflammation, chronic active | 2 (4%) | 1 (2%) | | |
| Inflammation, suppurative | | | 1 (2%) | |
| Seminal vesicle | (50) | (50) | (50) | (50) |
| Inflammation, suppurative | | | 1 (2%) | |
| Mineralization | | | 2 (4%) | 1 (2%) |
| Testes | (50) | (50) | (50) | (50) |
| Atrophy | 2 (4%) | 2 (4%) | 2 (4%) | |
| Interstitial cell, hyperplasia | 8 (16%) | 10 (20%) | 6 (12%) | 8 (16%) |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Thrombosis | 1 (2%) | | | |
| Lymph node | (11) | (17) | (16) | (17) |
| Iliac, pigmentation | | | | 1 (6%) |
| Lumbar, hemorrhage | | | | 2 (12%) |
| Pancreatic, edema | | | | 1 (6%) |
| Renal, edema | | | | 2 (12%) |
| Renal, hemorrhage | | | | 1 (6%) |
| Renal, pigmentation | | | | 1 (6%) |
| Lymph node, bronchial | (45) | (50) | (46) | (47) |
| Hemorrhage | | | | 1 (2%) |
| Lymph node, mandibular | (50) | (49) | (49) | (46) |
| Hemorrhage | | 2 (4%) | | |
| Infiltration cellular, plasma cell | 2 (4%) | | 5 (10%) | 2 (4%) |
| Lymph node, mesenteric | (50) | (50) | (50) | (50) |
| Edema | | 1 (2%) | | |
| Hemorrhage | | 1 (2%) | | 2 (4%) |
| Lymph node, mediastinal | (49) | (47) | (48) | (46) |
| Hemorrhage | 3 (6%) | 1 (2%) | | 3 (7%) |
| Inflammation, granulomatous | | 1 (2%) | 1 (2%) | |
| Spleen | (50) | (50) | (50) | (50) |
| Congestion | | 1 (2%) | | |
| Developmental malformation | 2 (4%) | 3 (6%) | 1 (2%) | 4 (8%) |
| Fibrosis | 9 (18%) | 14 (28%) | 11 (22%) | 6 (12%) |
| Hematopoietic cell proliferation | | 1 (2%) | 1 (2%) | 2 (4%) |
| Infiltration cellular, lipocyte | | 1 (2%) | | |
| Capsule, fibrosis | 1 (2%) | | 1 (2%) | |
| Thymus | (49) | (47) | (47) | (45) |
| Hemorrhage | 1 (2%) | | | |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|---|----------|----------|----------|----------|
| 2-Year Study (continued) | | | | |
| Integumentary System | | | | |
| Mammary gland | (33) | (36) | (41) | (34) |
| Galactocele | 1 (3%) | | 1 (2%) | |
| Inflammation, chronic active | 1 (3%) | 1 (3%) | | |
| Skin | (49) | (50) | (50) | (50) |
| Acanthosis | | | | 1 (2%) |
| Fibrosis | 1 (2%) | | | |
| Hemorrhage | | | 1 (2%) | |
| Hyperkeratosis | 1 (2%) | 1 (2%) | | 1 (2%) |
| Inflammation, chronic active | 9 (18%) | 4 (8%) | 4 (8%) | 4 (8%) |
| Necrosis | | | | 1 (2%) |
| Prepuce, edema | 1 (2%) | | | |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Hyperostosis | 1 (2%) | 1 (2%) | 1 (2%) | |
| Cranium, fibrous osteodystrophy | 1 (2%) | 1 (2%) | 3 (6%) | 17 (34%) |
| Femur, fibrous osteodystrophy | 1 (2%) | 1 (2%) | 3 (6%) | 19 (38%) |
| Sternum, developmental malformation | | | 1 (2%) | |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Compression | 12 (24%) | 11 (22%) | 9 (18%) | 6 (12%) |
| Hemorrhage | | 1 (2%) | 1 (2%) | |
| Respiratory System | | | | |
| Larynx | (50) | (50) | (50) | (50) |
| Inflammation, chronic active | 10 (20%) | 9 (18%) | 8 (16%) | 12 (24%) |
| Metaplasia, squamous | 1 (2%) | 1 (2%) | 1 (2%) | |
| Mineralization | 36 (72%) | 38 (76%) | 36 (72%) | 33 (66%) |
| Lung | (50) | (50) | (50) | (50) |
| Congestion | | | | 1 (2%) |
| Hemorrhage | 9 (18%) | 5 (10%) | 3 (6%) | 8 (16%) |
| Inflammation, chronic active | 1 (2%) | | | |
| Mineralization | 1 (2%) | 1 (2%) | 3 (6%) | 12 (24%) |
| Thrombosis | 1 (2%) | | | 1 (2%) |
| Alveolar epithelium, hyperplasia | 5 (10%) | 4 (8%) | 3 (6%) | 3 (6%) |
| Nose | (50) | (50) | (50) | (50) |
| Inflammation, suppurative | 5 (10%) | 3 (6%) | 4 (8%) | 7 (14%) |
| Thrombosis | 2 (4%) | | | 1 (2%) |
| Nasolacrimal duct, inflammation, chronic active | | | | 1 (2%) |
| Olfactory epithelium, degeneration | | | | 1 (2%) |
| Respiratory epithelium, hyperplasia | 1 (2%) | | | |
| Respiratory epithelium, metaplasia, squamous | 2 (4%) | | | |
| Trachea | (50) | (50) | (50) | (50) |
| Metaplasia, squamous | 1 (2%) | | | 1 (2%) |
| Mineralization | 1 (2%) | | 2 (4%) | 1 (2%) |

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

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--------------------------------------|----------|-----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Special Senses System | | | | |
| Eye | (45) | (42) | (48) | (42) |
| Cataract | 3 (7%) | | 4 (8%) | |
| Degeneration | | 1 (2%) | 4 (8%) | |
| Cornea, inflammation, chronic active | | 1 (2%) | | 4 (10%) |
| Cornea, mineralization | 1 (2%) | | 2 (4%) | 7 (17%) |
| Retina, atrophy | 3 (7%) | 2 (5%) | 5 (10%) | 2 (5%) |
| Harderian gland | | (1) | | |
| Hypertrophy | | 1 (100%) | | |
| Urinary System | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Developmental malformation | | | | 1 (2%) |
| Infarct | | 3 (6%) | | |
| Mineralization | | 1 (2%) | 3 (6%) | 4 (8%) |
| Nephropathy | 49 (98%) | 50 (100%) | 50 (100%) | 50 (100%) |
| Pelvis, dilatation | | | 1 (2%) | |
| Renal tubule, degeneration | 2 (4%) | 20 (40%) | 50 (100%) | 49 (98%) |
| Renal tubule, hyperplasia | 1 (2%) | 1 (2%) | 1 (2%) | 6 (12%) |
| Renal tubule, hyperplasia, oncocytic | | | 1 (2%) | |
| Urinary bladder | (49) | (50) | (50) | (50) |
| Hemorrhage | | | | 1 (2%) |
| Inflammation, suppurative | | | 1 (2%) | |
| Transitional epithelium, hyperplasia | | 1 (2%) | | |

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

| | | |
|------------------|---|------------|
| TABLE B1 | Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Tetrafluoroethylene | 127 |
| TABLE B2 | Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Tetrafluoroethylene | 132 |
| TABLE B3 | Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Tetrafluoroethylene | 150 |
| TABLE B4a | Historical Incidence of Renal Tubule Neoplasms in Chamber Control Female F344/N Rats | 157 |
| TABLE B4b | Historical Incidence of Liver Neoplasms in Chamber Control Female F344/N Rats | 157 |
| TABLE B4c | Historical Incidence of Mononuclear Cell Leukemia in Chamber Control Female F344/N Rats | 158 |
| TABLE B5 | Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Tetrafluoroethylene | 159 |

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Tetrafluoroethylene ^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|---------|---------|---------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation | 10 | 10 | 10 | 10 |
| Early deaths | | | | |
| Accidental death | | | | 1 |
| Moribund | 21 | 33 | 31 | 25 |
| Natural deaths | 1 | 1 | 4 | 6 |
| Survivors | | | | |
| Died last week of study | | 1 | | |
| Terminal sacrifice | 28 | 15 | 15 | 18 |
| Animals examined microscopically | 60 | 60 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Endocrine System | | | | |
| Pituitary gland | (10) | (10) | (10) | (10) |
| Pars distalis, adenoma | 1 (10%) | 2 (20%) | 1 (10%) | |
| Genital System | | | | |
| Clitoral gland | (10) | (10) | (10) | (10) |
| Adenoma | | 1 (10%) | | |
| Carcinoma | | 1 (10%) | | |
| Uterus | (10) | (10) | (10) | (10) |
| Polyp stromal | | | 2 (20%) | 1 (10%) |
| Hematopoietic System | | | | |
| Bone marrow | (10) | (10) | (10) | (10) |
| Histiocytic sarcoma | 1 (10%) | | | |
| Integumentary System | | | | |
| Mammary gland | (10) | (10) | (10) | (10) |
| Fibroadenoma | | | | 1 (10%) |
| Respiratory System | | | | |
| Lung | (10) | (10) | (10) | (10) |
| Histiocytic sarcoma, metastatic, bone marrow | 1 (10%) | | | |
| Urinary System | | | | |
| Kidney | (10) | (10) | (10) | (10) |
| Mixed tumor malignant | | | | 1 (10%) |
| Renal tubule, adenoma | | | | 1 (10%) |
| Systemic Lesions | | | | |
| Multiple organs ^b | (10) | (10) | (10) | (10) |
| Histiocytic sarcoma | 1 (10%) | | | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|----------|---------|---------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Systems Examined With No Neoplasms Observed | | | | |
| Alimentary System | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |
| Special Senses System | | | | |
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Intestine large, colon | (50) | (50) | (49) | (50) |
| Intestine large, rectum | (50) | (50) | (49) | (50) |
| Sarcoma stromal, metastatic, uterus | | 1 (2%) | | |
| Intestine large, cecum | (50) | (50) | (49) | (50) |
| Hemangiosarcoma, metastatic, liver | | | | 1 (2%) |
| Intestine small, duodenum | (49) | (50) | (49) | (50) |
| Intestine small, jejunum | (50) | (50) | (47) | (49) |
| Hemangiosarcoma, metastatic, liver | | | | 1 (2%) |
| Intestine small, ileum | (50) | (49) | (48) | (49) |
| Liver | (50) | (50) | (50) | (50) |
| Hemangiosarcoma | | | 5 (10%) | 1 (2%) |
| Hepatocellular carcinoma | | 4 (8%) | 6 (12%) | 2 (4%) |
| Hepatocellular carcinoma, multiple | | | 3 (6%) | |
| Hepatocellular adenoma | | 3 (6%) | 4 (8%) | 5 (10%) |
| Hepatocellular adenoma, multiple | | 1 (2%) | 1 (2%) | 1 (2%) |
| Sarcoma | | | 1 (2%) | |
| Mesentery | (6) | (4) | (4) | (6) |
| Leiomyosarcoma, metastatic, uterus | 1 (17%) | | | |
| Pancreas | (50) | (50) | (49) | (50) |
| Salivary glands | (50) | (50) | (50) | (50) |
| Adenoma | | 1 (2%) | | |
| Stomach, forestomach | (50) | (50) | (48) | (50) |
| Squamous cell carcinoma | 1 (2%) | | | |
| Stomach, glandular | (50) | (50) | (49) | (50) |
| Hemangiosarcoma, metastatic, liver | | | | 1 (2%) |
| Tongue | (2) | | (2) | (1) |
| Squamous cell papilloma | 2 (100%) | | | |
| Cardiovascular System | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Schwannoma NOS | 1 (2%) | | | |
| Endocrine System | | | | |
| Adrenal cortex | (50) | (50) | (50) | (50) |
| Hemangiosarcoma, metastatic, liver | | | 1 (2%) | |
| Leiomyosarcoma, metastatic, uterus | 1 (2%) | | | |
| Adrenal medulla | (50) | (50) | (50) | (50) |
| Pheochromocytoma benign | 4 (8%) | 1 (2%) | 2 (4%) | 5 (10%) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Endocrine System (continued) | | | | |
| Islets, pancreatic | (50) | (50) | (49) | (50) |
| Adenoma | | | 1 (2%) | |
| Carcinoma | | 1 (2%) | | |
| Parathyroid gland | (48) | (49) | (49) | (47) |
| Adenoma | 1 (2%) | | | |
| Pituitary gland | (50) | (50) | (49) | (50) |
| Pars distalis, adenoma | 43 (86%) | 34 (68%) | 36 (73%) | 34 (68%) |
| Pars distalis, carcinoma | | 1 (2%) | | |
| Thyroid gland | (50) | (50) | (50) | (50) |
| C-cell, adenoma | 5 (10%) | 9 (18%) | 5 (10%) | 2 (4%) |
| C-cell, carcinoma | 2 (4%) | 2 (4%) | 3 (6%) | 2 (4%) |
| Follicular cell, carcinoma | | 1 (2%) | | |
| General Body System | | | | |
| Tissue NOS | | | | (1) |
| Abdominal, fat, hemangiosarcoma, metastatic, tissue NOS | | | | 1 (100%) |
| Genital System | | | | |
| Clitoral gland | (49) | (49) | (50) | (48) |
| Adenoma | 6 (12%) | 6 (12%) | 2 (4%) | |
| Carcinoma | 1 (2%) | 1 (2%) | 2 (4%) | 2 (4%) |
| Ovary | (50) | (50) | (50) | (50) |
| Granulosa-theca tumor benign | | | 1 (2%) | 1 (2%) |
| Hepatocellular carcinoma, metastatic, liver | | | 1 (2%) | |
| Uterus | (50) | (50) | (50) | (50) |
| Leiomyosarcoma | 1 (2%) | 1 (2%) | | |
| Polyp stromal | 5 (10%) | 4 (8%) | 4 (8%) | 5 (10%) |
| Polyp stromal, multiple | 1 (2%) | 1 (2%) | | |
| Sarcoma stromal | | 1 (2%) | | 1 (2%) |
| Vagina | (1) | | (1) | (1) |
| Polyp | 1 (100%) | | | 1 (100%) |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | 1 (2%) | | | |
| Lymph node | (3) | (7) | (7) | (13) |
| Iliac, osteosarcoma, metastatic, bone | | | | 1 (8%) |
| Lymph node, bronchial | (50) | (50) | (50) | (48) |
| Lymph node, mandibular | (48) | (49) | (50) | (50) |
| Lymph node, mesenteric | (50) | (50) | (50) | (50) |
| Leiomyosarcoma, metastatic, uterus | 1 (2%) | | | |
| Lymph node, mediastinal | (49) | (49) | (49) | (45) |
| Spleen | (50) | (50) | (49) | (50) |
| Thymus | (47) | (48) | (49) | (48) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Integumentary System | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Adenoma | 1 (2%) | | 1 (2%) | 1 (2%) |
| Carcinoma | 3 (6%) | 1 (2%) | 3 (6%) | 1 (2%) |
| Carcinoma, multiple | | | 1 (2%) | |
| Fibroadenoma | 17 (34%) | 10 (20%) | 8 (16%) | 5 (10%) |
| Fibroadenoma, multiple | 5 (10%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Skin | (50) | (50) | (50) | (50) |
| Basosquamous tumor malignant | | | 1 (2%) | 1 (2%) |
| Keratoacanthoma | 2 (4%) | | | 1 (2%) |
| Squamous cell papilloma | | | 1 (2%) | 1 (2%) |
| Subcutaneous tissue, fibroma | | 1 (2%) | | |
| Subcutaneous tissue, neurofibroma | | | | 1 (2%) |
| Subcutaneous tissue, sarcoma | 1 (2%) | 1 (2%) | 1 (2%) | |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Vertebra, osteosarcoma | | | | 1 (2%) |
| Vertebra, sarcoma | 1 (2%) | | | |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Astrocytoma NOS | | 1 (2%) | | 1 (2%) |
| Respiratory System | | | | |
| Larynx | (50) | (50) | (50) | (50) |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | | | 1 (2%) | |
| Alveolar/bronchiolar carcinoma | | 1 (2%) | | |
| Carcinoma, metastatic, mammary gland | | | 1 (2%) | |
| Carcinoma, metastatic, pituitary gland | | 1 (2%) | | |
| Carcinoma, metastatic, thyroid gland | | 1 (2%) | | 1 (2%) |
| Hemangiosarcoma, metastatic, liver | | | 3 (6%) | |
| Hepatocellular carcinoma, metastatic, liver | | | 2 (4%) | |
| Leiomyosarcoma, metastatic, uterus | 1 (2%) | | | |
| Osteosarcoma, metastatic, bone | | | | 1 (2%) |
| Sarcoma, metastatic, bone | 1 (2%) | | | |
| Sarcoma, metastatic, liver | | | 1 (2%) | |
| Nose | (50) | (50) | (50) | (50) |
| Trachea | (50) | (50) | (50) | (50) |
| Special Senses System | | | | |
| Eye | (50) | (50) | (50) | (50) |
| Zymbal's gland | | | (1) | (1) |
| Adenoma | | | 1 (100%) | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|----------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Urinary System | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Leiomyosarcoma, metastatic, uterus | 1 (2%) | | | |
| Renal tubule, adenoma | | 3 (6%) | 1 (2%) | 3 (6%) |
| Renal tubule, carcinoma | | | | 2 (4%) |
| Urinary bladder | (49) | (50) | (50) | (49) |
| Sarcoma stromal, metastatic, uterus | | 1 (2%) | | |
| Transitional epithelium, papilloma | | | 2 (4%) | |
| Systemic Lesions | | | | |
| Multiple organs | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | 1 (2%) | | | |
| Leukemia mononuclear | 16 (32%) | 31 (62%) | 23 (46%) | 36 (72%) |
| Mesothelioma malignant | | 1 (2%) | | |
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | | | | |
| 15-Month interim evaluation | 1 | 4 | 3 | 3 |
| 2-Year study | 49 | 50 | 49 | 48 |
| Total primary neoplasms | | | | |
| 15-Month interim evaluation | 2 | 4 | 3 | 4 |
| 2-Year study | 121 | 123 | 121 | 118 |
| Total animals with benign neoplasms | | | | |
| 15-Month interim evaluation | 1 | 3 | 3 | 3 |
| 2-Year study | 46 | 43 | 43 | 40 |
| Total benign neoplasms | | | | |
| 15-Month interim evaluation | 1 | 3 | 3 | 3 |
| 2-Year study | 93 | 75 | 72 | 68 |
| Total animals with malignant neoplasms | | | | |
| 15-Month interim evaluation | 1 | 1 | | 1 |
| 2-Year study | 21 | 41 | 35 | 42 |
| Total malignant neoplasms | | | | |
| 15-Month interim evaluation | 1 | 1 | | 1 |
| 2-Year study | 27 | 47 | 49 | 49 |
| Total animals with metastatic neoplasms | | | | |
| 15-Month interim evaluation | 1 | | | |
| 2-Year study | 2 | 3 | 8 | 3 |
| Total metastatic neoplasms | | | | |
| 15-Month interim evaluation | 1 | | | |
| 2-Year study | 6 | 4 | 9 | 7 |
| Total animals with uncertain neoplasms- benign or malignant | | | | |
| 2-Year study | 1 | 1 | | 1 |
| Total uncertain neoplasms | | | | |
| 2-Year study | 1 | 1 | | 1 |

^a Number of animals examined microscopically at the site and the number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Tetrafluoroethylene: 0 ppm

| Number of Days on Study | 4 | 4 | 4 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | | |
|------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|--|
| | 3 | 8 | 8 | 5 | 8 | 2 | 2 | 4 | 4 | 6 | 7 | 8 | 9 | 9 | 9 | 9 | 0 | 0 | 0 | 1 | 2 | 2 | 3 | 3 | 3 | | |
| | 3 | 1 | 1 | 4 | 1 | 1 | 6 | 8 | 9 | 3 | 7 | 5 | 2 | 3 | 8 | 9 | 2 | 7 | 7 | 4 | 1 | 6 | 4 | 4 | 4 | | |
| Carcass ID Number | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | | |
| | 0 | 7 | 1 | 0 | 1 | 2 | 9 | 9 | 9 | 7 | 1 | 8 | 8 | 6 | 9 | 7 | 9 | 6 | 1 | 6 | 1 | 9 | 6 | 6 | 6 | | |
| | 2 | 3 | 5 | 7 | 2 | 0 | 9 | 3 | 2 | 1 | 7 | 0 | 3 | 3 | 4 | 0 | 5 | 7 | 8 | 6 | 3 | 8 | 1 | 2 | 4 | | |
| Alimentary System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine large, colon | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine large, rectum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine large, cecum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine small, duodenum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine small, jejunum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine small, ileum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Mesentery | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Leiomyosarcoma, metastatic, uterus | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Salivary glands | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Stomach, forestomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Squamous cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Stomach, glandular | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Tongue | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Cardiovascular System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Schwannoma NOS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Endocrine System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adrenal cortex | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Leiomyosarcoma, metastatic, uterus | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adrenal medulla | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Pheochromocytoma benign | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Islets, pancreatic | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Parathyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Pars distalis, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| C-cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C-cell, carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| General Body System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| None | | | | | | | | | | | | | | | | | | | | | | | | | | | |

+: Tissue examined microscopically
A: Autolysis precludes examination

M: Missing tissue
I: Insufficient tissue

X: Lesion present
Blank: Not examined

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|------------|------------|-----------|----------------|
| Adrenal Medulla: Benign Pheochromocytoma | | | | |
| Overall rate ^a | 4/50 (8%) | 1/50 (2%) | 2/50 (4%) | 5/50 (10%) |
| Adjusted rate ^b | 12.7% | 3.2% | 9.6% | 20.4% |
| Terminal rate ^c | 2/28 (7%) | 0/16 (0%) | 1/15 (7%) | 2/18 (11%) |
| First incidence (days) | 698 | 618 | 653 | 610 |
| Life table test ^d | P=0.136 | P=0.347N | P=0.577N | P=0.239 |
| Logistic regression test ^d | P=0.188 | P=0.247N | P=0.461N | P=0.354 |
| Cochran-Armitage test ^d | P=0.273 | | | |
| Fisher exact test ^d | | P=0.181N | P=0.339N | P=0.500 |
| Clitoral Gland: Adenoma | | | | |
| Overall rate | 6/49 (12%) | 6/49 (12%) | 2/50 (4%) | 0/48 (0%) |
| Adjusted rate | 18.7% | 31.4% | 11.9% | 0.0% |
| Terminal rate | 4/28 (14%) | 4/16 (25%) | 1/15 (7%) | 0/18 (0%) |
| First incidence (days) | 648 | 617 | 733 | — ^e |
| Life table test | P=0.029N | P=0.263 | P=0.365N | P=0.062N |
| Logistic regression test | P=0.020N | P=0.382 | P=0.242N | P=0.046N |
| Cochran-Armitage test | P=0.006N | | | |
| Fisher exact test | | P=0.620N | P=0.128N | P=0.014N |
| Clitoral Gland: Adenoma or Carcinoma | | | | |
| Overall rate | 7/49 (14%) | 7/49 (14%) | 4/50 (8%) | 2/48 (4%) |
| Adjusted rate | 22.1% | 37.1% | 19.5% | 8.6% |
| Terminal rate | 5/28 (18%) | 5/16 (31%) | 1/15 (7%) | 1/18 (6%) |
| First incidence (days) | 648 | 617 | 625 | 610 |
| Life table test | P=0.152N | P=0.223 | P=0.594N | P=0.243N |
| Logistic regression test | P=0.114N | P=0.333 | P=0.412N | P=0.180N |
| Cochran-Armitage test | P=0.042N | | | |
| Fisher exact test | | P=0.613N | P=0.251N | P=0.084N |
| Kidney (Renal Tubule): Adenoma (Single Sections) | | | | |
| Overall rate | 0/50 (0%) | 3/50 (6%) | 1/50 (2%) | 3/50 (6%) |
| Adjusted rate | 0.0% | 13.7% | 3.3% | 16.7% |
| Terminal rate | 0/28 (0%) | 1/16 (6%) | 0/15 (0%) | 3/18 (17%) |
| First incidence (days) | — | 574 | 668 | 734(T) |
| Life table test | P=0.095 | P=0.066 | P=0.443 | P=0.054 |
| Logistic regression test | P=0.099 | P=0.100 | P=0.505 | P=0.054 |
| Cochran-Armitage test | P=0.165 | | | |
| Fisher exact test | | P=0.121 | P=0.500 | P=0.121 |
| Kidney (Renal Tubule): Adenoma (Step Sections) | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 2/50 (4%) | 5/50 (10%) |
| Adjusted rate | 0.0% | 0.0% | 11.9% | 23.0% |
| Terminal rate | 0/28 (0%) | 0/16 (0%) | 1/15 (7%) | 3/18 (17%) |
| First incidence (days) | — | — | 733 | 628 |
| Life table test | P<0.001 | — | P=0.133 | P=0.008 |
| Logistic regression test | P<0.001 | — | P=0.143 | P=0.013 |
| Cochran-Armitage test | P=0.003 | | | |
| Fisher exact test | | — | P=0.247 | P=0.028 |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|-----------|------------|------------|-------------|
| Kidney (Renal Tubule): Adenoma (Single and Step Sections) | | | | |
| Overall rate | 0/50 (0%) | 3/50 (6%) | 3/50 (6%) | 8/50 (16%) |
| Adjusted rate | 0.0% | 13.7% | 14.8% | 38.4% |
| Terminal rate | 0/28 (0%) | 1/16 (6%) | 1/15 (7%) | 6/18 (33%) |
| First incidence (days) | — | 574 | 668 | 628 |
| Life table test | P<0.001 | P=0.066 | P=0.057 | P<0.001 |
| Logistic regression test | P<0.001 | P=0.100 | P=0.076 | P<0.001 |
| Cochran-Armitage test | P=0.002 | | | |
| Fisher exact test | | P=0.121 | P=0.121 | P=0.003 |
| Kidney (Renal Tubule): Adenoma or Carcinoma (Single Sections) | | | | |
| Overall rate | 0/50 (0%) | 3/50 (6%) | 1/50 (2%) | 5/50 (10%) |
| Adjusted rate | 0.0% | 13.7% | 3.3% | 27.8% |
| Terminal rate | 0/28 (0%) | 1/16 (6%) | 0/15 (0%) | 5/18 (28%) |
| First incidence (days) | — | 574 | 668 | 734 (T) |
| Life table test | P=0.012 | P=0.066 | P=0.443 | P=0.007 |
| Logistic regression test | P=0.010 | P=0.100 | P=0.505 | P=0.007 |
| Cochran-Armitage test | P=0.029 | | | |
| Fisher exact test | | P=0.121 | P=0.500 | P=0.028 |
| Kidney (Renal Tubule): Adenoma or Carcinoma (Single and Step Sections) | | | | |
| Overall rate | 0/50 (0%) | 3/50 (6%) | 3/50 (6%) | 10/50 (20%) |
| Adjusted rate | 0.0% | 13.7% | 14.8% | 48.8% |
| Terminal rate | 0/28 (0%) | 1/16 (6%) | 1/15 (7%) | 8/18 (44%) |
| First incidence (days) | — | 574 | 668 | 628 |
| Life table test | P<0.001 | P=0.066 | P=0.057 | P<0.001 |
| Logistic regression test | P<0.001 | P=0.100 | P=0.076 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P=0.121 | P=0.121 | P<0.001 |
| Liver: Hemangiosarcoma | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 5/50 (10%) | 1/50 (2%) |
| Adjusted rate | 0.0% | 0.0% | 19.5% | 5.0% |
| Terminal rate | 0/28 (0%) | 0/16 (0%) | 0/15 (0%) | 0/18 (0%) |
| First incidence (days) | — | — | 589 | 703 |
| Life table test | P=0.143 | — | P=0.013 | P=0.400 |
| Logistic regression test | P=0.182 | — | P=0.025 | P=0.435 |
| Cochran-Armitage test | P=0.242 | | | |
| Fisher exact test | | — | P=0.028 | P=0.500 |
| Liver: Hepatocellular Adenoma | | | | |
| Overall rate | 0/50 (0%) | 4/50 (8%) | 5/50 (10%) | 6/50 (12%) |
| Adjusted rate | 0.0% | 22.6% | 33.3% | 28.2% |
| Terminal rate | 0/28 (0%) | 3/16 (19%) | 5/15 (33%) | 3/18 (17%) |
| First incidence (days) | — | 707 | 734 (T) | 677 |
| Life table test | P=0.006 | P=0.017 | P=0.003 | P=0.003 |
| Logistic regression test | P=0.004 | P=0.020 | P=0.003 | P=0.003 |
| Cochran-Armitage test | P=0.026 | | | |
| Fisher exact test | | P=0.059 | P=0.028 | P=0.013 |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|-------------|-------------|-------------|------------|
| Liver: Hepatocellular Carcinoma | | | | |
| Overall rate | 0/50 (0%) | 4/50 (8%) | 9/50 (18%) | 2/50 (4%) |
| Adjusted rate | 0.0% | 20.9% | 48.1% | 11.1% |
| Terminal rate | 0/28 (0%) | 3/16 (19%) | 6/15 (40%) | 2/18 (11%) |
| First incidence (days) | — | 583 | 668 | 734 (T) |
| Life table test | P=0.146 | P=0.020 | P<0.001 | P=0.147 |
| Logistic regression test | P=0.128 | P=0.037 | P<0.001 | P=0.147 |
| Cochran-Armitage test | P=0.309 | | | |
| Fisher exact test | | P=0.059 | P=0.001 | P=0.247 |
| Liver: Hepatocellular Adenoma or Carcinoma | | | | |
| Overall rate | 0/50 (0%) | 7/50 (14%) | 12/50 (24%) | 8/50 (16%) |
| Adjusted rate | 0.0% | 36.3% | 65.4% | 37.8% |
| Terminal rate | 0/28 (0%) | 5/16 (31%) | 9/15 (60%) | 5/18 (28%) |
| First incidence (days) | — | 583 | 668 | 677 |
| Life table test | P=0.002 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.001 | P=0.002 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P=0.020 | | | |
| Fisher exact test | | P=0.006 | P<0.001 | P=0.003 |
| Mammary Gland: Fibroadenoma | | | | |
| Overall rate | 22/50 (44%) | 11/50 (22%) | 9/50 (18%) | 7/50 (14%) |
| Adjusted rate | 55.2% | 41.8% | 38.1% | 28.0% |
| Terminal rate | 11/28 (39%) | 5/16 (31%) | 4/15 (27%) | 3/18 (17%) |
| First incidence (days) | 481 | 468 | 534 | 504 |
| Life table test | P=0.033N | P=0.277N | P=0.162N | P=0.055N |
| Logistic regression test | P=0.003N | P=0.024N | P=0.009N | P=0.004N |
| Cochran-Armitage test | P=0.001N | | | |
| Fisher exact test | | P=0.016N | P=0.004N | P<0.001N |
| Mammary Gland: Fibroadenoma or Adenoma | | | | |
| Overall rate | 23/50 (46%) | 11/50 (22%) | 10/50 (20%) | 8/50 (16%) |
| Adjusted rate | 57.8% | 41.8% | 41.1% | 32.8% |
| Terminal rate | 12/28 (43%) | 5/16 (31%) | 4/15 (27%) | 4/18 (22%) |
| First incidence (days) | 481 | 468 | 534 | 504 |
| Life table test | P=0.050N | P=0.232N | P=0.196N | P=0.068N |
| Logistic regression test | P=0.005N | P=0.016N | P=0.011N | P=0.006N |
| Cochran-Armitage test | P=0.002N | | | |
| Fisher exact test | | P=0.010N | P=0.005N | P=0.001N |
| Mammary Gland: Carcinoma | | | | |
| Overall rate | 3/50 (6%) | 1/50 (2%) | 4/50 (8%) | 1/50 (2%) |
| Adjusted rate | 8.9% | 2.6% | 18.6% | 5.6% |
| Terminal rate | 1/28 (4%) | 0/16 (0%) | 2/15 (13%) | 1/18 (6%) |
| First incidence (days) | 693 | 581 | 611 | 734 (T) |
| Life table test | P=0.523N | P=0.461N | P=0.262 | P=0.501N |
| Logistic regression test | P=0.431N | P=0.318N | P=0.421 | P=0.461N |
| Cochran-Armitage test | P=0.343N | | | |
| Fisher exact test | | P=0.309N | P=0.500 | P=0.309N |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-------------|-------------|-------------|-------------|
| Mammary Gland: Adenoma or Carcinoma | | | | |
| Overall rate | 4/50 (8%) | 1/50 (2%) | 5/50 (10%) | 2/50 (4%) |
| Adjusted rate | 12.2% | 2.6% | 22.5% | 11.1% |
| Terminal rate | 2/28 (7%) | 0/16 (0%) | 2/15 (13%) | 2/18 (11%) |
| First incidence (days) | 693 | 581 | 611 | 734 (T) |
| Life table test | P=0.522 | P=0.336N | P=0.226 | P=0.575N |
| Logistic regression test | P=0.558N | P=0.213N | P=0.389 | P=0.565N |
| Cochran-Armitage test | P=0.420N | | | |
| Fisher exact test | | P=0.181N | P=0.500 | P=0.339N |
| Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma | | | | |
| Overall rate | 24/50 (48%) | 12/50 (24%) | 13/50 (26%) | 8/50 (16%) |
| Adjusted rate | 59.0% | 43.3% | 49.8% | 32.8% |
| Terminal rate | 12/28 (43%) | 5/16 (31%) | 5/15 (33%) | 4/18 (22%) |
| First incidence (days) | 481 | 468 | 534 | 504 |
| Life table test | P=0.050N | P=0.260N | P=0.386N | P=0.054N |
| Logistic regression test | P=0.004N | P=0.016N | P=0.037N | P=0.004N |
| Cochran-Armitage test | P=0.001N | | | |
| Fisher exact test | | P=0.011N | P=0.019N | P<0.001N |
| Pituitary Gland (Pars Distalis): Adenoma | | | | |
| Overall rate | 43/50 (86%) | 34/50 (68%) | 36/49 (73%) | 34/50 (68%) |
| Adjusted rate | 93.4% | 88.4% | 94.0% | 88.9% |
| Terminal rate | 25/28 (89%) | 12/16 (75%) | 13/15 (87%) | 14/18 (78%) |
| First incidence (days) | 481 | 413 | 387 | 433 |
| Life table test | P=0.177 | P=0.156 | P=0.074 | P=0.164 |
| Logistic regression test | P=0.270N | P=0.112N | P=0.253N | P=0.251N |
| Cochran-Armitage test | P=0.063N | | | |
| Fisher exact test | | P=0.028N | P=0.096N | P=0.028N |
| Pituitary Gland (Pars Distalis): Adenoma or Carcinoma | | | | |
| Overall rate | 43/50 (86%) | 35/50 (70%) | 36/49 (73%) | 34/50 (68%) |
| Adjusted rate | 93.4% | 91.3% | 94.0% | 88.9% |
| Terminal rate | 25/28 (89%) | 13/16 (81%) | 13/15 (87%) | 14/18 (78%) |
| First incidence (days) | 481 | 413 | 387 | 433 |
| Life table test | P=0.186 | P=0.112 | P=0.074 | P=0.164 |
| Logistic regression test | P=0.248N | P=0.176N | P=0.253N | P=0.251N |
| Cochran-Armitage test | P=0.052N | | | |
| Fisher exact test | | P=0.045N | P=0.096N | P=0.028N |
| Skin: Squamous Cell Papilloma, Keratoacanthoma, or Malignant Basosquamous Tumor | | | | |
| Overall rate | 2/50 (4%) | 0/50 (0%) | 2/50 (4%) | 3/50 (6%) |
| Adjusted rate | 7.1% | 0.0% | 11.1% | 16.7% |
| Terminal rate | 2/28 (7%) | 0/16 (0%) | 1/15 (7%) | 3/18 (17%) |
| First incidence (days) | 734 (T) | — | 702 | 734 (T) |
| Life table test | P=0.136 | P=0.368N | P=0.470 | P=0.301 |
| Logistic regression test | P=0.120 | P=0.368N | P=0.550 | P=0.301 |
| Cochran-Armitage test | P=0.237 | | | |
| Fisher exact test | | P=0.247N | P=0.691N | P=0.500 |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|------------|-------------|------------|------------|
| Thyroid Gland (C-cell): Adenoma | | | | |
| Overall rate | 5/50 (10%) | 9/50 (18%) | 5/50 (10%) | 2/50 (4%) |
| Adjusted rate | 16.7% | 35.0% | 22.7% | 8.2% |
| Terminal rate | 4/28 (14%) | 1/16 (6%) | 2/15 (13%) | 1/18 (6%) |
| First incidence (days) | 698 | 523 | 587 | 579 |
| Life table test | P=0.265N | P=0.044 | P=0.316 | P=0.420N |
| Logistic regression test | P=0.169N | P=0.084 | P=0.487 | P=0.370N |
| Cochran-Armitage test | P=0.086N | | | |
| Fisher exact test | | P=0.194 | P=0.630N | P=0.218N |
| Thyroid Gland (C-cell): Carcinoma | | | | |
| Overall rate | 2/50 (4%) | 2/50 (4%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate | 7.1% | 8.2% | 20.0% | 11.1% |
| Terminal rate | 2/28 (7%) | 1/16 (6%) | 3/15 (20%) | 2/18 (11%) |
| First incidence (days) | 734 (T) | 468 | 734 (T) | 734 (T) |
| Life table test | P=0.406 | P=0.536 | P=0.228 | P=0.528 |
| Logistic regression test | P=0.427 | P=0.701N | P=0.228 | P=0.528 |
| Cochran-Armitage test | P=0.569 | | | |
| Fisher exact test | | P=0.691N | P=0.500 | P=0.691N |
| Thyroid Gland (C-cell): Adenoma or Carcinoma | | | | |
| Overall rate | 7/50 (14%) | 11/50 (22%) | 7/50 (14%) | 4/50 (8%) |
| Adjusted rate | 23.6% | 40.6% | 34.6% | 19.0% |
| Terminal rate | 6/28 (21%) | 2/16 (13%) | 4/15 (27%) | 3/18 (17%) |
| First incidence (days) | 698 | 468 | 587 | 579 |
| Life table test | P=0.365N | P=0.041 | P=0.215 | P=0.548N |
| Logistic regression test | P=0.253N | P=0.110 | P=0.386 | P=0.543N |
| Cochran-Armitage test | P=0.117N | | | |
| Fisher exact test | | P=0.218 | P=0.613N | P=0.262N |
| Uterus: Stromal Polyp | | | | |
| Overall rate | 6/50 (12%) | 5/50 (10%) | 4/50 (8%) | 5/50 (10%) |
| Adjusted rate | 19.7% | 24.2% | 21.2% | 18.2% |
| Terminal rate | 5/28 (18%) | 3/16 (19%) | 2/15 (13%) | 2/18 (11%) |
| First incidence (days) | 626 | 583 | 699 | 439 |
| Life table test | P=0.432 | P=0.405 | P=0.538 | P=0.489 |
| Logistic regression test | P=0.552 | P=0.569 | P=0.564N | P=0.568N |
| Cochran-Armitage test | P=0.437N | | | |
| Fisher exact test | | P=0.500N | P=0.370N | P=0.500N |
| Uterus: Stromal Polyp or Stromal Sarcoma | | | | |
| Overall rate | 6/50 (12%) | 6/50 (12%) | 4/50 (8%) | 6/50 (12%) |
| Adjusted rate | 19.7% | 26.9% | 21.2% | 20.8% |
| Terminal rate | 5/28 (18%) | 3/16 (19%) | 2/15 (13%) | 2/18 (11%) |
| First incidence (days) | 626 | 583 | 699 | 439 |
| Life table test | P=0.328 | P=0.270 | P=0.538 | P=0.348 |
| Logistic regression test | P=0.468 | P=0.456 | P=0.564N | P=0.582 |
| Cochran-Armitage test | P=0.530N | | | |
| Fisher exact test | | P=0.620N | P=0.370N | P=0.620N |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-------------|-------------|--------------|-------------|
| All Organs: Hemangiosarcoma | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 5/50 (10%) | 1/50 (2%) |
| Adjusted rate | 0.0% | 0.0% | 19.5% | 5.0% |
| Terminal rate | 0/28 (0%) | 0/16 (0%) | 0/15 (0%) | 0/18 (0%) |
| First incidence (days) | — | — | 589 | 703 |
| Life table test | P=0.143 | — | P=0.013 | P=0.400 |
| Logistic regression test | P=0.182 | — | P=0.025 | P=0.435 |
| Cochran-Armitage test | P=0.242 | — | — | — |
| Fisher exact test | — | — | P=0.028 | P=0.500 |
| All Organs: Mononuclear Cell Leukemia | | | | |
| Overall rate | 16/50 (32%) | 31/50 (62%) | 23/50 (46%) | 36/50 (72%) |
| Adjusted rate | 43.7% | 76.5% | 65.1% | 82.6% |
| Terminal rate | 8/28 (29%) | 7/16 (44%) | 6/15 (40%) | 11/18 (61%) |
| First incidence (days) | 621 | 468 | 469 | 372 |
| Life table test | P<0.001 | P<0.001 | P=0.008 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P=0.105 | P<0.001 |
| Cochran-Armitage test | P<0.001 | — | — | — |
| Fisher exact test | — | P=0.002 | P=0.109 | P<0.001 |
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 46/50 (92%) | 43/50 (86%) | 43/50 (86%) | 40/50 (80%) |
| Adjusted rate | 95.8% | 97.6% | 100.0% | 95.1% |
| Terminal rate | 26/28 (93%) | 15/16 (94%) | 15/15 (100%) | 16/18 (89%) |
| First incidence (days) | 481 | 413 | 387 | 433 |
| Life table test | P=0.080 | P=0.016 | P=0.013 | P=0.057 |
| Logistic regression test | P=0.305N | P=0.546N | P=0.499N | P=0.360N |
| Cochran-Armitage test | P=0.065N | — | — | — |
| Fisher exact test | — | P=0.262N | P=0.262N | P=0.074N |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 21/50 (42%) | 41/50 (82%) | 35/50 (70%) | 42/50 (84%) |
| Adjusted rate | 52.7% | 93.0% | 84.0% | 91.1% |
| Terminal rate | 10/28 (36%) | 13/16 (81%) | 9/15 (60%) | 14/18 (78%) |
| First incidence (days) | 433 | 413 | 387 | 372 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P=0.005 | P<0.001 |
| Cochran-Armitage test | P<0.001 | — | — | — |
| Fisher exact test | — | P<0.001 | P=0.004 | P<0.001 |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-------------|--------------|--------------|-------------|
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 49/50 (98%) | 50/50 (100%) | 49/50 (98%) | 48/50 (96%) |
| Adjusted rate | 98.0% | 100.0% | 100.0% | 98.0% |
| Terminal rate | 27/28 (96%) | 16/16 (100%) | 15/15 (100%) | 17/18 (94%) |
| First incidence (days) | 433 | 247 | 387 | 372 |
| Life table test | P=0.021 | P=0.003 | P=0.004 | P=0.009 |
| Logistic regression test | P=0.526N | P=0.612 | P=0.629 | P=0.748 |
| Cochran-Armitage test | P=0.247N | | | |
| Fisher exact test | | P=0.500 | P=0.753N | P=0.500N |

(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, kidney, liver, pituitary gland, thyroid gland, and uterus; 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 B4a
Historical Incidence of Renal Tubule Neoplasms in Chamber Control Female F344/N Rats^a

| Study | Incidence in Controls | | |
|--|-----------------------|--------------|----------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence at Battelle Pacific Northwest Laboratories | | | |
| <i>o</i> -Chlorobenzalmalononitrile (CS-2) | 0/49 | 0/49 | 0/49 |
| Acetonitrile | 0/48 | 0/48 | 0/48 |
| α -Chloroacetophenone | 0/49 | 0/49 | 0/49 |
| Epinephrine Hydrochloride | 0/50 | 0/50 | 0/50 |
| Ethyl Chloride | 0/50 | 0/50 | 0/50 |
| Hexachlorocyclopentadiene | 0/50 | 1/50 | 1/50 |
| Ozone | 1/50 | 0/50 | 1/50 |
| Total | 1/346 (0.3%) | 1/346 (0.3%) | 2/346 (0.6%) |
| Standard deviation | 0.8% | 0.8% | 1.0% |
| Range | 0%-2% | 0%-2% | 0%-2% |
| Overall Historical Incidence | | | |
| Total | 1/650 (0.2%) | 1/650 (0.2%) | 2/650 (0.3%) |
| Standard deviation | 0.6% | 0.6% | 0.8% |
| Range | 0%-2% | 0%-2% | 0%-2% |

^a Data as of 12 May 1995

TABLE B4b
Historical Incidence of Liver Neoplasms in Chamber Control Female F344/N Rats^a

| Study | Incidence in Controls | | | |
|--|-----------------------|--------------|--------------|----------------------|
| | Hemangiosarcoma | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence at Battelle Pacific Northwest Laboratories | | | | |
| <i>o</i> -Chlorobenzalmalononitrile (CS-2) | 0/49 | 3/49 | 0/49 | 3/49 |
| Acetonitrile | 0/48 | 0/48 | 0/48 | 0/48 |
| α -Chloroacetophenone | 0/49 | 0/49 | 0/49 | 0/49 |
| Epinephrine Hydrochloride | 0/50 | 2/50 | 0/50 | 2/50 |
| Ethyl Chloride | 0/50 | 0/50 | 1/50 | 1/50 |
| Hexachlorocyclopentadiene | 0/50 | 1/50 | 0/50 | 1/50 |
| Ozone | 0/50 | 0/50 | 0/50 | 0/50 |
| Total | 0/346 | 6/346 (1.7%) | 1/346 (0.3%) | 7/346 (2.0%) |
| Standard deviation | | 2.4% | 0.8% | 2.3% |
| Range | | 0%-6% | 0%-2% | 0%-6% |
| Overall Historical Incidence | | | | |
| Total | 0/650 | 9/650 (1.4%) | 1/650 (0.2%) | 10/650 (1.5%) |
| Standard deviation | | 2.1% | 0.6% | 2.0% |
| Range | | 0%-6% | 0%-2% | 0%-6% |

^a Data as of 12 May 1995

TABLE B4c
Historical Incidence of Mononuclear Cell Leukemia in Chamber Control Female F344/N Rats ^a

| Study | Incidence in Controls |
|--|-----------------------|
| Historical Incidence at Battelle Pacific Northwest Laboratories | |
| <i>o</i> -Chlorobenzalmalononitrile (CS-2) | 24/50 |
| Acetonitrile | 18/48 |
| α -Chloroacetophenone | 27/50 |
| Epinephrine Hydrochloride | 24/50 |
| Ethyl Chloride | 20/50 |
| Hexachlorocyclopentadiene | 16/50 |
| Ozone | 17/50 |
| Total | 146/348 (42.0%) |
| Standard deviation | 8.2% |
| Range | 32%-54% |
| Overall Historical Incidence | |
| Total | 262/653 (40.1%) |
| Standard deviation | 7.2% |
| Range | 30%-54% |

^a Data as of 12 May 1995; includes data for lymphocytic, monocytic, and undifferentiated cell type leukemias

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|------------------------------------|----------|-----------|-----------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 60 | 60 | 60 | 60 |
| 15-Month interim evaluation | | | | |
| Early deaths | 10 | 10 | 10 | 10 |
| Accidental death | | | | 1 |
| Moribund | 21 | 33 | 31 | 25 |
| Natural deaths | 1 | 1 | 4 | 6 |
| Survivors | | | | |
| Died last week of study | | 1 | | |
| Terminal sacrifice | 28 | 15 | 15 | 18 |
| Animals examined microscopically | 60 | 60 | 60 | 60 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Liver | (10) | (10) | (10) | (10) |
| Angiectasis | | 1 (10%) | | |
| Basophilic focus | 9 (90%) | 9 (90%) | 10 (100%) | 10 (100%) |
| Clear cell focus | | 2 (20%) | 3 (30%) | 3 (30%) |
| Eosinophilic focus | | 5 (50%) | 2 (20%) | |
| Hepatodiaphragmatic nodule | | 2 (20%) | | 1 (10%) |
| Mixed cell focus | | 1 (10%) | 6 (60%) | 4 (40%) |
| Bile duct, hyperplasia | 1 (10%) | | | |
| Mesentery | (2) | (2) | (3) | (1) |
| Fat, necrosis | 2 (100%) | 2 (100%) | 3 (100%) | 1 (100%) |
| Pancreas | (10) | (10) | (10) | (10) |
| Metaplasia, hepatocyte | | | | 2 (20%) |
| Acinus, atrophy | 3 (30%) | 1 (10%) | 2 (20%) | 3 (30%) |
| Stomach, forestomach | (10) | (10) | (10) | (10) |
| Acanthosis | | | 1 (10%) | |
| Ulcer | | | 1 (10%) | |
| Cardiovascular System | | | | |
| Heart | (10) | (10) | (10) | (10) |
| Cardiomyopathy | 8 (80%) | 10 (100%) | 9 (90%) | 10 (100%) |
| Endocrine System | | | | |
| Adrenal cortex | (10) | (10) | (10) | (10) |
| Hyperplasia | 1 (10%) | 1 (10%) | 1 (10%) | 2 (20%) |
| Hypertrophy | 2 (20%) | | | |
| Parathyroid gland | (10) | (10) | (10) | (10) |
| Hyperplasia | | | | 1 (10%) |
| Pituitary gland | (10) | (10) | (10) | (10) |
| Pars distalis, hyperplasia | 6 (60%) | 5 (50%) | 8 (80%) | 7 (70%) |
| Thyroid gland | (10) | (10) | (10) | (10) |
| C-cell, hyperplasia | 9 (90%) | 7 (70%) | 9 (90%) | 9 (90%) |

^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 Tetrafluoroethylene (continued)

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-----------|-----------|-----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Genital System | | | | |
| Clitoral gland | (10) | (10) | (10) | (10) |
| Inflammation, chronic active | 1 (10%) | | 1 (10%) | |
| Ovary | (10) | (10) | (10) | (10) |
| Cyst | | 1 (10%) | 1 (10%) | |
| Hematopoietic System | | | | |
| Bone marrow | (10) | (10) | (10) | (10) |
| Hyperplasia, reticulum cell | 2 (20%) | 2 (20%) | 3 (30%) | 2 (20%) |
| Lymph node | | (1) | | |
| Renal, hemorrhage | | 1 (100%) | | |
| Lymph node, mediastinal | (8) | (10) | (10) | (9) |
| Hemorrhage | 3 (38%) | 2 (20%) | 1 (10%) | |
| Spleen | (10) | (10) | (10) | (10) |
| Developmental malformation | | 1 (10%) | | |
| Respiratory System | | | | |
| Larynx | (10) | (10) | (10) | (10) |
| Inflammation, chronic active | 2 (20%) | 3 (30%) | 5 (50%) | 3 (30%) |
| Metaplasia, squamous | | 2 (20%) | 1 (10%) | 1 (10%) |
| Mineralization | 7 (70%) | 6 (60%) | 10 (100%) | 10 (100%) |
| Lung | (10) | (10) | (10) | (10) |
| Hemorrhage | 10 (100%) | 10 (100%) | 10 (100%) | 10 (100%) |
| Infiltration cellular, histiocyte | 1 (10%) | 1 (10%) | 3 (30%) | 1 (10%) |
| Inflammation, chronic active | | | 1 (10%) | |
| Alveolar epithelium, hyperplasia | 1 (10%) | 1 (10%) | 2 (20%) | |
| Nose | (10) | (10) | (10) | (10) |
| Inflammation, chronic active | | | | 1 (10%) |
| Special Senses System | | | | |
| Eye | (1) | | | |
| Cataract | 1 (100%) | | | |
| Retina, atrophy | 1 (100%) | | | |
| Urinary System | | | | |
| Kidney | (10) | (10) | (10) | (10) |
| Nephropathy | 8 (80%) | 9 (90%) | 9 (90%) | 10 (100%) |
| Renal tubule, degeneration | | | 10 (100%) | 10 (100%) |
| Renal tubule, hyperplasia | | | 1 (10%) | |
| Renal tubule, hyperplasia, oncocytic | | | | 1 (10%) |
| Urinary bladder | (10) | (10) | (10) | (10) |
| Transitional epithelium, hyperplasia | | | | 1 (10%) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|----------|----------|----------|-----------|
| <i>15-Month Interim Evaluation</i> (continued) | | | | |
| <i>Systems Examined With No Lesions Observed</i> | | | | |
| General Body System | | | | |
| Integumentary System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |
| | | | | |
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Intestine large, colon | (50) | (50) | (49) | (50) |
| Cyst | | | 1 (2%) | |
| Parasite metazoan | 4 (8%) | 5 (10%) | 3 (6%) | 3 (6%) |
| Intestine large, rectum | (50) | (50) | (49) | (50) |
| Diverticulum | 1 (2%) | | | |
| Parasite metazoan | 5 (10%) | 2 (4%) | 2 (4%) | 6 (12%) |
| Intestine large, cecum | (50) | (50) | (49) | (50) |
| Diverticulum | 1 (2%) | | | |
| Fibrosis | | | | 1 (2%) |
| Infiltration cellular, eosinophil | 1 (2%) | | | |
| Parasite metazoan | 1 (2%) | 1 (2%) | | 4 (8%) |
| Intestine small, duodenum | (49) | (50) | (49) | (50) |
| Necrosis | | | 1 (2%) | |
| Liver | (50) | (50) | (50) | (50) |
| Angiectasis | | 9 (18%) | 9 (18%) | 14 (28%) |
| Basophilic focus | 41 (82%) | 38 (76%) | 41 (82%) | 37 (74%) |
| Clear cell focus | 10 (20%) | 3 (6%) | 12 (24%) | 9 (18%) |
| Degeneration, cystic | 1 (2%) | 4 (8%) | 1 (2%) | 3 (6%) |
| Embolus bacterial | | | | 1 (2%) |
| Eosinophilic focus | 1 (2%) | 4 (8%) | 5 (10%) | 4 (8%) |
| Fatty change | 15 (30%) | 20 (40%) | 17 (34%) | 10 (20%) |
| Hepatodiaphragmatic nodule | 3 (6%) | 5 (10%) | 10 (20%) | 5 (10%) |
| Mitotic alteration | | | 1 (2%) | |
| Mixed cell focus | 12 (24%) | 14 (28%) | 16 (32%) | 18 (36%) |
| Necrosis, focal | 1 (2%) | | 2 (4%) | |
| Regeneration | 1 (2%) | 2 (4%) | 3 (6%) | 8 (16%) |
| Bile duct, hyperplasia | 9 (18%) | 18 (36%) | 12 (24%) | 18 (36%) |
| Centrilobular, necrosis | | | 2 (4%) | 2 (4%) |
| Mesentery | (6) | (4) | (4) | (6) |
| Polyarteritis | 1 (17%) | | 1 (25%) | |
| Fat, necrosis | 4 (67%) | 4 (100%) | 4 (100%) | 4 (67%) |
| Pancreas | (50) | (50) | (49) | (50) |
| Basophilic focus | 3 (6%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Hyperplasia | 1 (2%) | | | |
| Metaplasia | | 1 (2%) | | |
| Acinus, atrophy | 15 (30%) | 14 (28%) | 9 (18%) | 9 (18%) |
| Pharynx | | (1) | | |
| Palate, hyperplasia | | 1 (100%) | | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|----------|--------------------|-----------|
| 2-Year Study (continued) | | | | |
| Alimentary System (continued) | | | | |
| Stomach, forestomach | (50) | (50) | (48) | (50) |
| Acanthosis | 4 (8%) | 4 (8%) | 1 (2%) | 1 (2%) |
| Diverticulum | | 1 (2%) | | |
| Inflammation, suppurative | | | 1 (2%) | |
| Mineralization | | 1 (2%) | | |
| Necrosis | 5 (10%) | 4 (8%) | | |
| Stomach, glandular | (50) | (50) | (49) | (50) |
| Mineralization | 1 (2%) | | 1 (2%) | 2 (4%) |
| Necrosis | 2 (4%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Tongue | (2) | | (2) | (1) |
| Hyperplasia, squamous Epithelium, hyperplasia | | | 1 (50%) 1 (50%) | 1 (100%) |
| Cardiovascular System | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Cardiomyopathy | 41 (82%) | 40 (80%) | 40 (80%) | 33 (66%) |
| Embolus bacterial | | | | 1 (2%) |
| Polyarteritis | 1 (2%) | | | |
| Atrium, thrombosis | 1 (2%) | 2 (4%) | | 1 (2%) |
| Endocrine System | | | | |
| Adrenal cortex | (50) | (50) | (50) | (50) |
| Embolus bacterial | | | | 1 (2%) |
| Fibrosis | | 1 (2%) | | |
| Hyperplasia | 18 (36%) | 11 (22%) | 16 (32%) | 22 (44%) |
| Hypertrophy | 4 (8%) | 4 (8%) | 3 (6%) | 5 (10%) |
| Metaplasia, osseous | | | 1 (2%) | |
| Necrosis | | | 1 (2%) | |
| Vacuolization cytoplasmic | 5 (10%) | 3 (6%) | 4 (8%) | 3 (6%) |
| Adrenal medulla | (50) | (50) | (50) | (50) |
| Embolus bacterial | | | | 1 (2%) |
| Hyperplasia | 6 (12%) | 9 (18%) | 3 (6%) | 8 (16%) |
| Islets, pancreatic | (50) | (50) | (49) | (50) |
| Hyperplasia | | 1 (2%) | | |
| Pituitary gland | (50) | (50) | (49) | (50) |
| Pars distalis, angiectasis | 1 (2%) | | | |
| Pars distalis, hemorrhage | 1 (2%) | | | |
| Pars distalis, hyperplasia | 4 (8%) | 8 (16%) | 8 (16%) | 13 (26%) |
| Thyroid gland | (50) | (50) | (50) | (50) |
| C-cell, hyperplasia | 48 (96%) | 40 (80%) | 41 (82%) | 38 (76%) |
| Follicular cell, hyperplasia | 2 (4%) | | 1 (2%) | |
| General Body System | | | | |
| None | | | | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|------------------------------------|---------|----------|---------|-----------|
| 2-Year Study (continued) | | | | |
| Genital System | | | | |
| Clitoral gland | (49) | (49) | (50) | (48) |
| Dilatation | 1 (2%) | | | |
| Hyperplasia | 1 (2%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Inflammation, chronic active | 2 (4%) | 4 (8%) | 7 (14%) | 2 (4%) |
| Duct, ectasia | 1 (2%) | | | |
| Ovary | (50) | (50) | (50) | (50) |
| Cyst | 4 (8%) | 3 (6%) | 2 (4%) | 2 (4%) |
| Uterus | (50) | (50) | (50) | (50) |
| Cyst | 1 (2%) | | | 1 (2%) |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Hyperplasia, reticulum cell | | | | 1 (2%) |
| Myelofibrosis | | 3 (6%) | 1 (2%) | |
| Lymph node | (3) | (7) | (7) | (13) |
| Lumbar, pigmentation | | 1 (14%) | | |
| Renal, hemorrhage | 1 (33%) | | 1 (14%) | 2 (15%) |
| Lymph node, bronchial | (50) | (50) | (50) | (48) |
| Hemorrhage | | | 2 (4%) | |
| Lymph node, mandibular | (48) | (49) | (50) | (50) |
| Hemorrhage | 1 (2%) | 2 (4%) | | |
| Infiltration cellular, plasma cell | | | 3 (6%) | 2 (4%) |
| Lymph node, mesenteric | (50) | (50) | (50) | (50) |
| Embolus bacterial | | | | 1 (2%) |
| Hemorrhage | | 1 (2%) | 1 (2%) | |
| Lymph node, mediastinal | (49) | (49) | (49) | (45) |
| Hemorrhage | 1 (2%) | 3 (6%) | 2 (4%) | 2 (4%) |
| Spleen | (50) | (50) | (49) | (50) |
| Angiectasis | | | 1 (2%) | 1 (2%) |
| Fibrosis | 1 (2%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Hematopoietic cell proliferation | | | 1 (2%) | |
| Infiltration cellular, lipocyte | | 2 (4%) | | |
| Thymus | (47) | (48) | (49) | (48) |
| Cyst | | | | 1 (2%) |
| Integumentary System | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Cyst | | | | 1 (2%) |
| Hyperplasia, focal | | 1 (2%) | | |
| Skin | (50) | (50) | (50) | (50) |
| Inflammation, chronic active | | 2 (4%) | 4 (8%) | 1 (2%) |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Hyperostosis | 6 (12%) | 14 (28%) | 8 (16%) | 3 (6%) |
| Tibia, fracture | | | | 1 (2%) |
| Turbinates, fracture | | | | 1 (2%) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|----------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Compression | 30 (60%) | 23 (46%) | 20 (40%) | 19 (38%) |
| Embolus bacterial | | | | 1 (2%) |
| Hemorrhage | | 2 (4%) | | |
| Respiratory System | | | | |
| Larynx | (50) | (50) | (50) | (50) |
| Inflammation, chronic active | 13 (26%) | 14 (28%) | 14 (28%) | 21 (42%) |
| Metaplasia, squamous | 2 (4%) | 1 (2%) | 2 (4%) | 4 (8%) |
| Mineralization | 31 (62%) | 29 (58%) | 31 (62%) | 30 (60%) |
| Lung | (50) | (50) | (50) | (50) |
| Hemorrhage | 17 (34%) | 8 (16%) | 12 (24%) | 5 (10%) |
| Infiltration cellular, lymphocyte | 1 (2%) | | | 2 (4%) |
| Infiltration cellular, histiocyte | 2 (4%) | | | 1 (2%) |
| Inflammation, chronic active | 2 (4%) | 3 (6%) | | 1 (2%) |
| Metaplasia, osseous | | | | 1 (2%) |
| Alveolar epithelium, hyperplasia | 8 (16%) | 4 (8%) | 9 (18%) | 6 (12%) |
| Mediastinum, polyarteritis | 1 (2%) | | | |
| Nose | (50) | (50) | (50) | (50) |
| Inflammation, chronic active | | 1 (2%) | | |
| Inflammation, suppurative | 1 (2%) | 2 (4%) | 3 (6%) | 5 (10%) |
| Thrombosis | | | | 1 (2%) |
| Nares, inflammation, chronic active | | | | 1 (2%) |
| Nasolacrimal duct, inflammation, suppurative | 1 (2%) | | | |
| Special Senses System | | | | |
| Eye | (50) | (50) | (50) | (50) |
| Cataract | 15 (30%) | 4 (8%) | 10 (20%) | 45 (90%) |
| Degeneration | 2 (4%) | 1 (2%) | 3 (6%) | 2 (4%) |
| Hemorrhage | 3 (6%) | 3 (6%) | 2 (4%) | 3 (6%) |
| Cornea, inflammation, chronic active | 1 (2%) | 2 (4%) | | |
| Cornea, mineralization | 1 (2%) | 2 (4%) | 2 (4%) | 3 (6%) |
| Retina, atrophy | 21 (42%) | 11 (22%) | 15 (30%) | 21 (42%) |
| Retina, degeneration | 33 (66%) | 23 (46%) | 14 (28%) | 13 (26%) |
| Harderian gland | | (1) | | |
| Inflammation, chronic active | | 1 (100%) | | |
| Urinary System | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Embolus bacterial | | | | 1 (2%) |
| Hyperplasia, atypical | | | | 1 (2%) |
| Nephropathy | 48 (96%) | 46 (92%) | 48 (96%) | 47 (94%) |
| Papilla, necrosis | | 1 (2%) | | |
| Pelvis, dilatation | | 1 (2%) | | 1 (2%) |
| Renal tubule, degeneration | | | 35 (70%) | 46 (92%) |
| Renal tubule, hyperplasia | 1 (2%) | 3 (6%) | 6 (12%) | 12 (24%) |
| Renal tubule, hyperplasia, oncocytic | | | | 3 (6%) |
| Urinary bladder | (49) | (50) | (50) | (49) |
| Transitional epithelium, hyperplasia | 1 (2%) | 1 (2%) | | |

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

| | | |
|------------------|---|------------|
| TABLE C1 | Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Tetrafluoroethylene | 167 |
| TABLE C2 | Individual Animal Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Tetrafluoroethylene | 172 |
| TABLE C3 | Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of Tetrafluoroethylene | 194 |
| TABLE C4a | Historical Incidence of Liver Neoplasms in Chamber Control Male B6C3F₁ Mice | 199 |
| TABLE C4b | Historical Incidence of Histiocytic Sarcoma in Chamber Control Male B6C3F₁ Mice | 200 |
| TABLE C5 | Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Tetrafluoroethylene | 201 |

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of Tetrafluoroethylene ^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|----------|---------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 58 | 58 | 58 | 58 |
| 15-Month interim evaluation | 10 | 10 | 10 | 10 |
| Early deaths | | | | |
| Moribund | 6 | 24 | 26 | 27 |
| Natural deaths | 4 | 13 | 20 | 20 |
| Survivors | | | | |
| Terminal sacrifice | 38 | 11 | 2 | 1 |
| Animals examined microscopically | 58 | 58 | 58 | 58 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Liver | (10) | (10) | (10) | (10) |
| Hemangiosarcoma | | | | 3 (30%) |
| Hepatocellular carcinoma | 2 (20%) | 4 (40%) | 2 (20%) | 2 (20%) |
| Hepatocellular adenoma | 5 (50%) | 2 (20%) | 2 (20%) | 1 (10%) |
| Hepatocellular adenoma, multiple | 1 (10%) | | 2 (20%) | |
| Histiocytic sarcoma | | | | 1 (10%) |
| Stomach, forestomach | (10) | (10) | (10) | (10) |
| Squamous cell papilloma | | | 1 (10%) | |
| Hematopoietic System | | | | |
| Thymus | (8) | (9) | (10) | (9) |
| Thymoma NOS | | 1 (11%) | | |
| Respiratory System | | | | |
| Lung | (10) | (10) | (10) | (10) |
| Alveolar/bronchiolar adenoma | 1 (10%) | | | 1 (10%) |
| Hepatocellular carcinoma, metastatic, liver | 1 (10%) | | 1 (10%) | |
| Histiocytic sarcoma | | | | 1 (10%) |
| Special Senses System | | | | |
| Harderian gland | (1) | (2) | | (1) |
| Adenoma | 1 (100%) | 2 (100%) | | 1 (100%) |
| Systemic Lesions | | | | |
| Multiple organs ^b | (10) | (10) | (10) | (10) |
| Histiocytic sarcoma | | | | 1 (10%) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|----------|----------|----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Systems Examined With No Neoplasms Observed | | | | |
| Cardiovascular System | | | | |
| Endocrine System | | | | |
| General Body System | | | | |
| Genital System | | | | |
| Integumentary System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |
| Urinary System | | | | |
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Liver | (48) | (48) | (48) | (48) |
| Carcinoma, metastatic, harderian gland | | | | 1 (2%) |
| Carcinoma, metastatic, lung | | 1 (2%) | | |
| Hemangioma | | 3 (6%) | 3 (6%) | 1 (2%) |
| Hemangioma, multiple | | 7 (15%) | 2 (4%) | 1 (2%) |
| Hemangiosarcoma | | 5 (10%) | 10 (21%) | 19 (40%) |
| Hemangiosarcoma, multiple | | 16 (33%) | 17 (35%) | 18 (38%) |
| Hepatocellular carcinoma | 7 (15%) | 11 (23%) | 24 (50%) | 20 (42%) |
| Hepatocellular carcinoma, multiple | 4 (8%) | 9 (19%) | 9 (19%) | 6 (13%) |
| Hepatocellular adenoma | 11 (23%) | 11 (23%) | 5 (10%) | 13 (27%) |
| Hepatocellular adenoma, multiple | 6 (13%) | 6 (13%) | 7 (15%) | 7 (15%) |
| Histiocytic sarcoma | | 12 (25%) | 7 (15%) | 7 (15%) |
| Mesentery | (2) | (3) | (2) | (7) |
| Carcinoma, metastatic, lung | | 1 (33%) | | |
| Hemangioma | | | | 1 (14%) |
| Hemangiosarcoma | | | | 1 (14%) |
| Hepatocellular carcinoma, metastatic, liver | | | | 1 (14%) |
| Histiocytic sarcoma | | | 1 (50%) | |
| Pancreas | (48) | (48) | (46) | (45) |
| Hepatocellular carcinoma, metastatic, liver | | | | 1 (2%) |
| Stomach, forestomach | (48) | (48) | (46) | (46) |
| Squamous cell carcinoma | | | | 1 (2%) |
| Squamous cell papilloma | 2 (4%) | 1 (2%) | | 1 (2%) |
| Cardiovascular System | | | | |
| Heart | (48) | (48) | (48) | (48) |
| Carcinoma, metastatic, lung | | 1 (2%) | | 1 (2%) |
| Endocrine System | | | | |
| Adrenal cortex | (48) | (48) | (47) | (48) |
| Adenoma | 1 (2%) | | 1 (2%) | |
| Islets, pancreatic | (48) | (48) | (46) | (45) |
| Adenoma | | | | 1 (2%) |
| Thyroid gland | (48) | (48) | (47) | (47) |
| Follicular cell, adenoma | | 3 (6%) | | |
| Follicular cell, carcinoma | | 1 (2%) | | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|--------|----------|---------|-----------|
| 2-Year Study (continued) | | | | |
| General Body System | | | | |
| Tissue NOS | (1) | (2) | (2) | |
| Carcinoma, metastatic, lung | | 1 (50%) | | |
| Hemangioma | | 1 (50%) | | |
| Genital System | | | | |
| Epididymis | (48) | (48) | (48) | (48) |
| Leiomyoma | | | | 1 (2%) |
| Leiomyosarcoma | | 1 (2%) | | |
| Hematopoietic System | | | | |
| Bone marrow | (48) | (48) | (47) | (47) |
| Hemangioma | | | 1 (2%) | |
| Histiocytic sarcoma | | 1 (2%) | 1 (2%) | 2 (4%) |
| Lymph node | (3) | (1) | (2) | |
| Renal, histiocytic sarcoma | | 1 (100%) | 1 (50%) | |
| Lymph node, bronchial | (36) | (36) | (35) | (26) |
| Histiocytic sarcoma | | 3 (8%) | | 1 (4%) |
| Lymph node, mandibular | (36) | (34) | (32) | (34) |
| Histiocytic sarcoma | | 2 (6%) | 1 (3%) | |
| Lymph node, mesenteric | (47) | (42) | (41) | (40) |
| Histiocytic sarcoma | | 4 (10%) | 1 (2%) | 2 (5%) |
| Lymph node, mediastinal | (30) | (34) | (32) | (29) |
| Carcinoma, metastatic, lung | | 1 (3%) | | 1 (3%) |
| Histiocytic sarcoma | | 2 (6%) | | 1 (3%) |
| Spleen | (48) | (48) | (46) | (46) |
| Hemangiosarcoma | 2 (4%) | | | |
| Histiocytic sarcoma | | 2 (4%) | 1 (2%) | 2 (4%) |
| Thymus | (43) | (40) | (34) | (37) |
| Histiocytic sarcoma | | | | 1 (3%) |
| Integumentary System | | | | |
| Skin | (48) | (48) | (48) | (48) |
| Subcutaneous tissue, hemangioma | | 1 (2%) | | |
| Subcutaneous tissue, hemangiosarcoma | | | 1 (2%) | 1 (2%) |
| Musculoskeletal System | | | | |
| Bone | (48) | (48) | (48) | (48) |
| Carcinoma, metastatic, harderian gland | | | | 1 (2%) |
| Skeletal muscle | | | | (1) |
| Carcinoma, metastatic, lung | | | | 1 (100%) |
| Nervous System | | | | |
| Brain | (48) | (48) | (48) | (48) |
| Carcinoma, metastatic, harderian gland | | | | 1 (2%) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|---------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Respiratory System | | | | |
| Lung | (48) | (48) | (48) | (48) |
| Alveolar/bronchiolar adenoma | 9 (19%) | 7 (15%) | 12 (25%) | 3 (6%) |
| Alveolar/bronchiolar adenoma, multiple | 1 (2%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Alveolar/bronchiolar carcinoma | 2 (4%) | 4 (8%) | 3 (6%) | 1 (2%) |
| Carcinoma | | 1 (2%) | | 1 (2%) |
| Carcinoma, metastatic, harderian gland | 1 (2%) | | | 1 (2%) |
| Hemangiosarcoma, metastatic, liver | | | 2 (4%) | 2 (4%) |
| Hepatocellular carcinoma, metastatic, liver | 1 (2%) | 7 (15%) | 14 (29%) | 8 (17%) |
| Histiocytic sarcoma | | 7 (15%) | 4 (8%) | 3 (6%) |
| Nose | (48) | (47) | (46) | (47) |
| Carcinoma, metastatic, harderian gland | 1 (2%) | | | |
| Special Senses System | | | | |
| Harderian gland | (6) | (8) | (5) | (6) |
| Adenoma | 5 (83%) | 7 (88%) | 4 (80%) | 4 (67%) |
| Carcinoma | 1 (17%) | 1 (13%) | | 1 (17%) |
| Urinary System | | | | |
| Kidney | (48) | (48) | (48) | (48) |
| Carcinoma, metastatic, lung | | | | 1 (2%) |
| Hemangiosarcoma, metastatic, liver | | | 1 (2%) | |
| Histiocytic sarcoma | | 3 (6%) | 3 (6%) | 2 (4%) |
| Urinary bladder | (48) | (43) | (43) | (42) |
| Systemic Lesions | | | | |
| Multiple organs | (48) | (48) | (48) | (48) |
| Histiocytic sarcoma | | 12 (25%) | 7 (15%) | 7 (15%) |
| Lymphoma malignant mixed | 2 (4%) | 1 (2%) | | 1 (2%) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-------|---------|---------|-----------|
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | | | | |
| 15-Month interim evaluation | 6 | 7 | 6 | 6 |
| 2-Year study | 37 | 44 | 48 | 46 |
| Total primary neoplasms | | | | |
| 15-Month interim evaluation | 10 | 9 | 7 | 9 |
| 2-Year study | 53 | 110 | 107 | 112 |
| Total animals with benign neoplasms | | | | |
| 15-Month interim evaluation | 6 | 4 | 4 | 3 |
| 2-Year study | 26 | 30 | 27 | 27 |
| Total benign neoplasms | | | | |
| 15-Month interim evaluation | 8 | 4 | 5 | 3 |
| 2-Year study | 35 | 48 | 36 | 35 |
| Total animals with malignant neoplasms | | | | |
| 15-Month interim evaluation | 2 | 4 | 2 | 4 |
| 2-Year study | 16 | 39 | 47 | 46 |
| Total malignant neoplasms | | | | |
| 15-Month interim evaluation | 2 | 4 | 2 | 6 |
| 2-Year study | 18 | 62 | 71 | 77 |
| Total animals with metastatic neoplasms | | | | |
| 15-Month interim evaluation | 1 | | 1 | |
| 2-Year study | 2 | 8 | 14 | 11 |
| Total metastatic neoplasms | | | | |
| 15-Month interim evaluation | 1 | | 1 | |
| 2-Year study | 3 | 12 | 17 | 20 |
| Total animals with uncertain neoplasms- benign or malignant | | | | |
| 15-Month interim evaluation | | 1 | | |
| Total uncertain neoplasms | | | | |
| 15-Month interim evaluation | | 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 Tumor Pathology of Male Mice in the 2-Year Inhalation Study of Tetrafluoroethylene: 625 ppm (continued)

| | |
|---|---|
| Number of Days on Study | 3 3 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 |
| | 6 6 1 2 3 4 4 8 8 9 0 1 1 1 1 3 3 4 4 4 4 4 5 5 |
| | 3 8 5 0 3 6 7 4 5 7 9 6 7 8 8 1 9 1 1 1 7 8 8 1 4 |
| Carcass ID Number | 2 |
| | 7 8 5 6 5 8 5 6 3 4 7 6 4 8 8 5 3 4 5 8 7 5 7 4 5 |
| | 8 2 5 2 8 7 9 1 4 1 9 4 4 1 6 6 3 0 0 4 6 1 0 2 2 |
| Hematopoietic System | |
| Bone marrow | + + + + + + + + + + + + + + + + + + + A + + + + |
| Hemangioma | |
| Histiocytic sarcoma | |
| Lymph node | |
| Renal, histiocytic sarcoma | |
| Lymph node, bronchial | A M M + + + M + + + + + + I + + M M + + M + + + |
| Lymph node, mandibular | M + + + + + + + + + + + M + M M + M M + M + + + |
| Histiocytic sarcoma | |
| Lymph node, mesenteric | A + + + + + M + + + A + + + M M + + + + A + + + + |
| Histiocytic sarcoma | |
| Lymph node, mediastinal | A + + + + + M I + + M I M M M + M + + + + M + M + |
| Spleen | A + + + + + + + + + + + + + + + + + + A + + + + |
| Histiocytic sarcoma | |
| Thymus | A M + + + M M M M + M + M M + + + + + + M M M + |
| Integumentary System | |
| Mammary gland | M |
| Skin | + |
| Subcutaneous tissue, hemangiosarcoma | |
| Musculoskeletal System | |
| Bone | + |
| Nervous System | |
| Brain | + |
| Respiratory System | |
| Larynx | A + + + + + + + + + + + + + + + + + + A + + + + |
| Lung | + |
| Alveolar/bronchiolar adenoma | |
| Alveolar/bronchiolar adenoma, multiple | X X X |
| Alveolar/bronchiolar carcinoma | |
| Hemangiosarcoma, metastatic, liver | |
| Hepatocellular carcinoma, metastatic, liver | |
| Histiocytic sarcoma | |
| Nose | A + + + + + + + + + + + + + + + + + + A + + + + |
| Trachea | A + + + + + + + + + + + + + + + + + + A + + + + |
| Special Senses System | |
| Eye | |
| Adenoma | |
| Harderian gland | |
| Adenoma | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|----------------|-------------|-------------|-------------|
| Harderian Gland: Adenoma | | | | |
| Overall rate ^a | 5/48 (10%) | 7/48 (15%) | 4/48 (8%) | 4/48 (8%) |
| Adjusted rate ^b | 12.6% | 35.3% | 13.8% | 24.7% |
| Terminal rate ^c | 4/38 (11%) | 2/11 (18%) | 0/2 (0%) | 0/1 (0%) |
| First incidence (days) | 597 | 463 | 447 | 465 |
| Life table test ^d | P=0.047 | P=0.022 | P=0.100 | P=0.052 |
| Logistic regression test ^d | P=0.381N | P=0.239 | P=0.481N | P=0.545N |
| Cochran-Armitage test ^d | P=0.316N | | | |
| Fisher exact test ^d | | P=0.379 | P=0.500N | P=0.500N |
| Harderian Gland: Adenoma or Carcinoma | | | | |
| Overall rate | 6/48 (13%) | 8/48 (17%) | 4/48 (8%) | 5/48 (10%) |
| Adjusted rate | 14.6% | 42.5% | 13.8% | 26.6% |
| Terminal rate | 4/38 (11%) | 3/11 (27%) | 0/2 (0%) | 0/1 (0%) |
| First incidence (days) | 597 | 463 | 447 | 465 |
| Life table test | P=0.033 | P=0.016 | P=0.166 | P=0.058 |
| Logistic regression test | P=0.389N | P=0.230 | P=0.319N | P=0.487N |
| Cochran-Armitage test | P=0.312N | | | |
| Fisher exact test | | P=0.387 | P=0.370N | P=0.500N |
| Liver: Hemangioma | | | | |
| Overall rate | 0/48 (0%) | 10/48 (21%) | 5/48 (10%) | 2/48 (4%) |
| Adjusted rate | 0.0% | 53.2% | 60.1% | 12.3% |
| Terminal rate | 0/38 (0%) | 4/11 (36%) | 1/2 (50%) | 0/1 (0%) |
| First incidence (days) | — ^e | 590 | 548 | 599 |
| Life table test | P=0.012 | P<0.001 | P<0.001 | P=0.066 |
| Logistic regression test | P=0.289N | P<0.001 | P=0.015 | P=0.188 |
| Cochran-Armitage test | P=0.448N | | | |
| Fisher exact test | | P<0.001 | P=0.028 | P=0.247 |
| Liver: Hemangiosarcoma | | | | |
| Overall rate | 0/48 (0%) | 21/48 (44%) | 27/48 (56%) | 37/48 (77%) |
| Adjusted rate | 0.0% | 89.3% | 95.6% | 100.0% |
| Terminal rate | 0/38 (0%) | 9/11 (82%) | 1/2 (50%) | 1/1 (100%) |
| First incidence (days) | — | 522 | 497 | 401 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |
| Liver: Hemangioma or Hemangiosarcoma | | | | |
| Overall rate | 0/48 (0%) | 26/48 (54%) | 30/48 (63%) | 38/48 (79%) |
| Adjusted rate | 0.0% | 95.7% | 100.0% | 100.0% |
| Terminal rate | 0/38 (0%) | 10/11 (91%) | 2/2 (100%) | 1/1 (100%) |
| First incidence (days) | — | 522 | 497 | 401 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|-------------|-------------|-------------|-------------|
| Liver: Hepatocellular Adenoma | | | | |
| Overall rate | 17/48 (35%) | 17/48 (35%) | 12/48 (25%) | 20/48 (42%) |
| Adjusted rate | 42.1% | 83.5% | 70.3% | 71.3% |
| Terminal rate | 15/38 (39%) | 8/11 (73%) | 1/2 (50%) | 0/1 (0%) |
| First incidence (days) | 452 | 566 | 485 | 401 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.043 | P=0.059 | P=0.444N | P=0.181 |
| Cochran-Armitage test | P=0.322 | | | |
| Fisher exact test | | P=0.584N | P=0.187N | P=0.338 |
| Liver: Hepatocellular Carcinoma | | | | |
| Overall rate | 11/48 (23%) | 20/48 (42%) | 33/48 (69%) | 26/48 (54%) |
| Adjusted rate | 24.4% | 63.4% | 94.3% | 100.0% |
| Terminal rate | 5/38 (13%) | 3/11 (27%) | 1/2 (50%) | 1/1 (100%) |
| First incidence (days) | 475 | 462 | 363 | 315 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.150 | P=0.056 | P=0.055 | P=0.245 |
| Cochran-Armitage test | P=0.001 | | | |
| Fisher exact test | | P=0.040 | P<0.001 | P=0.002 |
| Liver: Hepatocellular Adenoma or Carcinoma | | | | |
| Overall rate | 26/48 (54%) | 34/48 (71%) | 39/48 (81%) | 35/48 (73%) |
| Adjusted rate | 57.4% | 96.8% | 100.0% | 100.0% |
| Terminal rate | 19/38 (50%) | 10/11 (91%) | 2/2 (100%) | 1/1 (100%) |
| First incidence (days) | 452 | 462 | 363 | 315 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.236 | P=0.015 | P=0.289 | P=0.550N |
| Cochran-Armitage test | P=0.040 | | | |
| Fisher exact test | | P=0.070 | P=0.004 | P=0.045 |
| Lung: Alveolar/bronchiolar Adenoma | | | | |
| Overall rate | 10/48 (21%) | 8/48 (17%) | 13/48 (27%) | 5/48 (10%) |
| Adjusted rate | 24.7% | 41.7% | 76.8% | 23.1% |
| Terminal rate | 8/38 (21%) | 3/11 (27%) | 1/2 (50%) | 0/1 (0%) |
| First incidence (days) | 475 | 522 | 433 | 548 |
| Life table test | P=0.007 | P=0.079 | P<0.001 | P=0.042 |
| Logistic regression test | P=0.297N | P=0.597 | P=0.324 | P=0.344N |
| Cochran-Armitage test | P=0.159N | | | |
| Fisher exact test | | P=0.397N | P=0.317 | P=0.130N |
| Lung: Alveolar/bronchiolar Carcinoma | | | | |
| Overall rate | 2/48 (4%) | 5/48 (10%) | 3/48 (6%) | 2/48 (4%) |
| Adjusted rate | 5.3% | 28.4% | 51.9% | 20.0% |
| Terminal rate | 2/38 (5%) | 2/11 (18%) | 0/2 (0%) | 0/1 (0%) |
| First incidence (days) | 666 (T) | 537 | 548 | 606 |
| Life table test | P=0.014 | P=0.015 | P=0.004 | P=0.043 |
| Logistic regression test | P=0.355 | P=0.103 | P=0.150 | P=0.330 |
| Cochran-Armitage test | P=0.420N | | | |
| Fisher exact test | | P=0.218 | P=0.500 | P=0.692N |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-------------|-------------|-------------|-------------|
| Lung: Alveolar/bronchiolar Adenoma or Carcinoma | | | | |
| Overall rate | 12/48 (25%) | 13/48 (27%) | 16/48 (33%) | 7/48 (15%) |
| Adjusted rate | 29.7% | 61.8% | 88.8% | 38.5% |
| Terminal rate | 10/38 (26%) | 5/11 (45%) | 1/2 (50%) | 0/1 (0%) |
| First incidence (days) | 475 | 522 | 433 | 548 |
| Life table test | P<0.001 | P=0.003 | P<0.001 | P=0.004 |
| Logistic regression test | P=0.383N | P=0.218 | P=0.136 | P=0.537N |
| Cochran-Armitage test | P=0.130N | | | |
| Fisher exact test | | P=0.500 | P=0.250 | P=0.153N |
| Thyroid Gland (Follicular Cell): Adenoma | | | | |
| Overall rate | 0/48 (0%) | 3/48 (6%) | 0/47 (0%) | 0/47 (0%) |
| Adjusted rate | 0.0% | 23.3% | 0.0% | 0.0% |
| Terminal rate | 0/38 (0%) | 2/11 (18%) | 0/2 (0%) | 0/1 (0%) |
| First incidence (days) | — | 637 | — | — |
| Life table test | P=0.345 | P=0.008 | — | — |
| Logistic regression test | P=0.590 | P=0.020 | — | — |
| Cochran-Armitage test | P=0.316N | | | |
| Fisher exact test | | P=0.121 | — | — |
| Thyroid Gland (Follicular Cell): Adenoma or Carcinoma | | | | |
| Overall rate | 0/48 (0%) | 4/48 (8%) | 0/47 (0%) | 0/47 (0%) |
| Adjusted rate | 0.0% | 31.8% | 0.0% | 0.0% |
| Terminal rate | 0/38 (0%) | 3/11 (27%) | 0/2 (0%) | 0/1 (0%) |
| First incidence (days) | — | 637 | — | — |
| Life table test | P=0.228 | P=0.001 | — | — |
| Logistic regression test | P=0.478 | P=0.004 | — | — |
| Cochran-Armitage test | P=0.252N | | | |
| Fisher exact test | | P=0.059 | — | — |
| All Organs: Hemangioma | | | | |
| Overall rate | 0/48 (0%) | 11/48 (23%) | 6/48 (13%) | 3/48 (6%) |
| Adjusted rate | 0.0% | 54.7% | 61.3% | 26.9% |
| Terminal rate | 0/38 (0%) | 4/11 (36%) | 1/2 (50%) | 0/1 (0%) |
| First incidence (days) | — | 566 | 541 | 599 |
| Life table test | P=0.005 | P<0.001 | P<0.001 | P=0.005 |
| Logistic regression test | P=0.311N | P<0.001 | P=0.011 | P=0.057 |
| Cochran-Armitage test | P=0.563N | | | |
| Fisher exact test | | P<0.001 | P=0.013 | P=0.121 |
| All Organs: Hemangiosarcoma | | | | |
| Overall rate | 2/48 (4%) | 21/48 (44%) | 27/48 (56%) | 37/48 (77%) |
| Adjusted rate | 5.3% | 89.3% | 95.6% | 100.0% |
| Terminal rate | 2/38 (5%) | 9/11 (82%) | 1/2 (50%) | 1/1 (100%) |
| First incidence (days) | 666 (T) | 522 | 497 | 401 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-------------|--------------|-------------|-------------|
| All Organs: Hemangioma or Hemangiosarcoma | | | | |
| Overall rate | 2/48 (4%) | 27/48 (56%) | 30/48 (63%) | 38/48 (79%) |
| Adjusted rate | 5.3% | 95.8% | 100.0% | 100.0% |
| Terminal rate | 2/38 (5%) | 10/11 (91%) | 2/2 (100%) | 1/1 (100%) |
| First incidence (days) | 666 (T) | 522 | 497 | 401 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |
| All Organs: Histiocytic Sarcoma | | | | |
| Overall rate | 0/48 (0%) | 12/48 (25%) | 7/48 (15%) | 7/48 (15%) |
| Adjusted rate | 0.0% | 47.8% | 35.1% | 65.8% |
| Terminal rate | 0/38 (0%) | 1/11 (9%) | 0/2 (0%) | 0/1 (0%) |
| First incidence (days) | — | 513 | 415 | 527 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.100 | P<0.001 | P=0.039 | P=0.004 |
| Cochran-Armitage test | P=0.143 | | | |
| Fisher exact test | | P<0.001 | P=0.006 | P=0.006 |
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 26/48 (54%) | 30/48 (63%) | 27/48 (56%) | 27/48 (56%) |
| Adjusted rate | 61.6% | 100.0% | 100.0% | 100.0% |
| Terminal rate | 22/38 (58%) | 11/11 (100%) | 2/2 (100%) | 1/1 (100%) |
| First incidence (days) | 452 | 463 | 433 | 401 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.135 | P=0.015 | P=0.329 | P=0.179 |
| Cochran-Armitage test | P=0.520N | | | |
| Fisher exact test | | P=0.267 | P=0.500 | P=0.500 |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 16/48 (33%) | 39/48 (81%) | 47/48 (98%) | 46/48 (96%) |
| Adjusted rate | 34.6% | 94.9% | 100.0% | 100.0% |
| Terminal rate | 8/38 (21%) | 9/11 (82%) | 2/2 (100%) | 1/1 (100%) |
| First incidence (days) | 475 | 462 | 363 | 315 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-------------|--------------|--------------|-------------|
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 37/48 (77%) | 44/48 (92%) | 48/48 (100%) | 46/48 (96%) |
| Adjusted rate | 78.6% | 100.0% | 100.0% | 100.0% |
| Terminal rate | 28/38 (74%) | 11/11 (100%) | 2/2 (100%) | 1/1 (100%) |
| First incidence (days) | 452 | 462 | 363 | 315 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.012 | P=0.002 | P=0.171 | P=0.001 |
| Cochran-Armitage test | P=0.002 | | | |
| Fisher exact test | | P=0.045 | P<0.001 | P=0.007 |

(T)Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, 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 C4a
Historical Incidence of Liver Neoplasms in Chamber Control Male B6C3F₁ Mice^a

| Study | Incidence in Controls | | | | |
|--|-----------------------|-----------------|------------------------|--------------------------|-------------------------------------|
| | Hemangioma | Hemangiosarcoma | Hepatocellular Adenoma | Hepatocellular Carcinoma | Hepatocellular Adenoma or Carcinoma |
| Historical Incidence at Battelle Pacific Northwest Laboratories | | | | | |
| 1,3-Butadiene | 0/50 | 0/50 | 13/50 | 11/50 | 21/50 |
| Acetonitrile | 0/50 | 1/50 | 13/50 | 7/50 | 19/50 |
| Allyl Glycidyl Ether | 0/49 | 0/49 | 15/49 | 10/49 | 23/49 |
| α-Chloroacetophenone | 0/50 | 0/50 | 5/50 | 11/50 | 16/50 |
| Epinephrine Hydrochloride | 0/50 | 1/50 | 10/50 | 12/50 | 20/50 |
| Ethyl Chloride | 0/50 | 0/50 | 6/50 | 9/50 | 15/50 |
| Hexachlorocyclopentadiene | 0/50 | 0/50 | 19/50 | 7/50 | 24/50 |
| o-Chlorobenzalmalononitrile (CS-2) | 1/49 | 0/49 | 4/49 | 14/49 | 18/49 |
| Ozone | 0/50 | 0/50 | 23/50 | 12/50 | 30/50 |
| Total | 1/448 (0.2%) | 2/448 (0.5%) | 108/448 (24.1%) | 93/448 (20.8%) | 186/448 (41.5%) |
| Standard deviation | 0.7% | 0.9% | 13.0% | 4.9% | 9.2% |
| Range | 0%-2% | 0%-2% | 8%-46% | 14%-29% | 30%-60% |
| Overall Historical Incidence | | | | | |
| Total | 2/947 (0.2%) | 12/947 (1.3%) | 200/947 (21.1%) | 184/947 (19.4%) | 358/947 (37.8%) |
| Standard deviation | 0.7% | 1.7% | 11.6% | 5.8% | 12.5% |
| Range | 0%-2% | 0%-6% | 4%-46% | 9%-29% | 11%-60% |

^a Data as of 12 May 1995

TABLE C4b
Historical Incidence of Histiocytic Sarcoma in Chamber Control Male B6C3F₁ Mice^a

| Study | Incidence in Controls |
|--|-----------------------|
| Historical Incidence at Battelle Pacific Northwest Laboratories | |
| 1,3-Butadiene | 0/50 |
| Acetonitrile | 0/50 |
| Allyl Glycidyl Ether | 0/50 |
| α -Chloroacetophenone | 0/50 |
| Epinephrine Hydrochloride | 1/50 |
| Ethyl Chloride | 0/50 |
| Hexachlorocyclopentadiene | 0/50 |
| <i>o</i> -Chlorobenzalmalononitrile (CS-2) | 1/50 |
| Ozone | 0/50 |
| Total | 2/450 (0.4%) |
| Standard deviation | 0.9% |
| Range | 0%-2% |
| Overall Historical Incidence | |
| Total | 6/950 (0.6%) |
| Standard deviation | 1.2% |
| Range | 0%-4% |

^a Data as of 12 May 1995

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|---------|---------|---------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 58 | 58 | 58 | 58 |
| 15-Month interim evaluation | | | | |
| Early deaths | | | | |
| Moribund | 6 | 24 | 26 | 27 |
| Natural deaths | 4 | 13 | 20 | 20 |
| Survivors | | | | |
| Terminal sacrifice | 38 | 11 | 2 | 1 |
| Animals examined microscopically | 58 | 58 | 58 | 58 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Intestine large, colon | (10) | (9) | (10) | (10) |
| Diverticulum | 1 (10%) | | | |
| Inflammation, chronic | 1 (10%) | | | |
| Liver | (10) | (10) | (10) | (10) |
| Angiectasis | | 1 (10%) | 5 (50%) | 2 (20%) |
| Basophilic focus | | | 1 (10%) | 1 (10%) |
| Clear cell focus | | | 2 (20%) | 1 (10%) |
| Eosinophilic focus | | | 3 (30%) | |
| Fatty change | 4 (40%) | 1 (10%) | 1 (10%) | 1 (10%) |
| Granuloma | | | | 1 (10%) |
| Hematopoietic cell proliferation | | | 1 (10%) | |
| Infarct | | | | 1 (10%) |
| Infiltration cellular, mixed cell | | | 1 (10%) | 2 (20%) |
| Mixed cell focus | 1 (10%) | 1 (10%) | 3 (30%) | 3 (30%) |
| Necrosis, coagulative, multifocal | 1 (10%) | 1 (10%) | | 1 (10%) |
| Salivary glands | (10) | (10) | (10) | (10) |
| Infiltration cellular, mononuclear cell | 4 (40%) | 2 (20%) | 1 (10%) | 3 (30%) |
| Stomach, forestomach | (10) | (10) | (10) | (10) |
| Epithelium, hyperplasia | | | | 1 (10%) |
| Tooth | | | | (1) |
| Inflammation, suppurative | | | | 1 (100%) |
| Endocrine System | | | | |
| Adrenal cortex | (10) | (10) | (10) | (10) |
| Capsule, hyperplasia | 6 (60%) | 4 (40%) | 7 (70%) | 2 (20%) |
| Islets, pancreatic | (10) | (10) | (10) | (10) |
| Hyperplasia | | | 1 (10%) | 1 (10%) |
| Thyroid gland | (10) | (10) | (10) | (10) |
| Ultimobranchial cyst | | | 1 (10%) | |
| Genital System | | | | |
| Preputial gland | (10) | (10) | (10) | (10) |
| Ectasia | | 4 (40%) | 1 (10%) | 1 (10%) |
| Infiltration cellular, mononuclear cell | 1 (10%) | 2 (20%) | 1 (10%) | |
| Inflammation, suppurative | | | 3 (30%) | |

^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 Tetrafluoroethylene (continued)

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|---------|---------|---------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Hematopoietic System | | | | |
| Bone marrow | (10) | (10) | (10) | (10) |
| Hyperplasia, neutrophil | | 1 (10%) | 2 (20%) | 1 (10%) |
| Lymph node, mandibular | (8) | (3) | (6) | (8) |
| Hyperplasia, plasma cell | | | | 1 (13%) |
| Spleen | (10) | (10) | (10) | (10) |
| Hematopoietic cell proliferation | 2 (20%) | 4 (40%) | 5 (50%) | 3 (30%) |
| Infiltration cellular, polymorphonuclear | | | 1 (10%) | |
| Integumentary System | | | | |
| Skin | (10) | (9) | (10) | (10) |
| Inflammation, chronic | | 1 (11%) | | |
| Musculoskeletal System | | | | |
| Bone | (10) | (10) | (10) | (10) |
| Sternum, fracture healed | | | 1 (10%) | |
| Nervous System | | | | |
| Brain | (10) | (10) | (10) | (10) |
| Mineralization | 5 (50%) | | 2 (20%) | 6 (60%) |
| Respiratory System | | | | |
| Larynx | (10) | (10) | (10) | (10) |
| Exudate, mucopurulent | | | | 1 (10%) |
| Lung | (10) | (10) | (10) | (10) |
| Alveolus, hemorrhage, multifocal | 1 (10%) | | | |
| Urinary System | | | | |
| Kidney | (10) | (10) | (10) | (10) |
| Infiltration cellular, mononuclear cell | 1 (10%) | | | |
| Inflammation, chronic active | | | 1 (10%) | |
| Nephropathy | 3 (30%) | 3 (30%) | | 3 (30%) |
| Bilateral, pelvis, dilatation | | 1 (10%) | 1 (10%) | |
| Renal tubule, dilatation | | | 6 (60%) | 10 (100%) |
| Renal tubule, karyomegaly | | | 4 (40%) | 10 (100%) |
| Urinary bladder | (10) | (10) | (10) | (10) |
| Inflammation, chronic active | | | 1 (10%) | |
| Systems Examined With No Lesions Observed | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| Special Senses System | | | | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|---------|---------|----------|-----------|
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Gallbladder | (42) | (35) | (31) | (27) |
| Inflammation, chronic active | | | | 1 (4%) |
| Intestine small, jejunum | (46) | (40) | (34) | (34) |
| Peyer's patch, hyperplasia, lymphoid | 1 (2%) | 1 (3%) | | |
| Intestine small, ileum | (46) | (39) | (35) | (36) |
| Peyer's patch, inflammation, chronic active | 1 (2%) | | | |
| Liver | (48) | (48) | (48) | (48) |
| Angiectasis | | 6 (13%) | 10 (21%) | 13 (27%) |
| Basophilic focus | 1 (2%) | 1 (2%) | 4 (8%) | 3 (6%) |
| Clear cell focus | 1 (2%) | | | |
| Eosinophilic focus | 1 (2%) | 6 (13%) | 7 (15%) | 7 (15%) |
| Fatty change | 2 (4%) | | | |
| Hematopoietic cell proliferation | 1 (2%) | 2 (4%) | 4 (8%) | |
| Hemorrhage | | 1 (2%) | | 1 (2%) |
| Infarct | 1 (2%) | 6 (13%) | 2 (4%) | 4 (8%) |
| Infiltration cellular, mixed cell | 1 (2%) | 5 (10%) | 3 (6%) | 3 (6%) |
| Mixed cell focus | 2 (4%) | 2 (4%) | 3 (6%) | |
| Necrosis, coagulative, multifocal | 4 (8%) | 3 (6%) | 13 (27%) | 11 (23%) |
| Thrombosis | | 1 (2%) | 3 (6%) | |
| Bile duct, hyperplasia | | 1 (2%) | | |
| Centrilobular, necrosis | | | 1 (2%) | 1 (2%) |
| Hepatocyte, regeneration | | | | 1 (2%) |
| Mesentery | (2) | (3) | (2) | (7) |
| Angiectasis | | | | 1 (14%) |
| Fat, hemorrhage | | | | 2 (29%) |
| Fat, necrosis | 1 (50%) | 2 (67%) | 1 (50%) | 3 (43%) |
| Pancreas | (48) | (48) | (46) | (45) |
| Granuloma | 1 (2%) | | | |
| Duct, ectasia | | | 1 (2%) | |
| Salivary glands | (48) | (48) | (48) | (48) |
| Infiltration cellular, mononuclear cell | 7 (15%) | 7 (15%) | | 3 (6%) |
| Stomach, forestomach | (48) | (48) | (46) | (46) |
| Foreign body | 1 (2%) | | | |
| Granuloma | 1 (2%) | | 1 (2%) | |
| Hemorrhage | 1 (2%) | | | |
| Hyperplasia, squamous | | | 1 (2%) | |
| Inflammation, focal | 1 (2%) | | | |
| Ulcer | | 1 (2%) | | 1 (2%) |
| Epithelium, hyperplasia | 2 (4%) | 3 (6%) | 2 (4%) | 3 (7%) |
| Serosa, inflammation | 1 (2%) | | | |
| Stomach, glandular | (48) | (48) | (46) | (45) |
| Foreign body | | | | 1 (2%) |
| Necrosis | | | | 2 (4%) |
| Tooth | (2) | (2) | (4) | (7) |
| Dysplasia | 1 (50%) | 1 (50%) | | 3 (43%) |
| Inflammation, suppurative | 1 (50%) | | 4 (100%) | 4 (57%) |
| Periodontal tissue, proliferation connective tissue | | 1 (50%) | | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Cardiovascular System | | | | |
| Heart | (48) | (48) | (48) | (48) |
| Cardiomyopathy | 2 (4%) | | 2 (4%) | |
| Fibrosis | | | | 1 (2%) |
| Infiltration cellular, mixed cell | | | 1 (2%) | |
| Atrium, thrombosis | 1 (2%) | | | |
| Endocrine System | | | | |
| Adrenal cortex | (48) | (48) | (47) | (48) |
| Accessory adrenal cortical nodule | 1 (2%) | | | |
| Cyst | | 1 (2%) | | |
| Hyperplasia | 1 (2%) | 3 (6%) | | |
| Capsule, hyperplasia | 29 (60%) | 26 (54%) | 24 (51%) | 20 (42%) |
| Islets, pancreatic | (48) | (48) | (46) | (45) |
| Hyperplasia | 2 (4%) | 1 (2%) | 7 (15%) | 2 (4%) |
| Pituitary gland | (47) | (44) | (44) | (44) |
| Pars distalis, cyst | 1 (2%) | 1 (2%) | 1 (2%) | |
| Pars distalis, hyperplasia | | 1 (2%) | | |
| Pars intermedia, hemorrhage | | 1 (2%) | | |
| Thyroid gland | (48) | (48) | (47) | (47) |
| Ultimobranchial cyst | | 1 (2%) | 1 (2%) | |
| Follicle, cyst | 1 (2%) | 1 (2%) | 1 (2%) | |
| Follicular cell, hyperplasia | | 1 (2%) | 1 (2%) | |
| General Body System | | | | |
| Tissue NOS | (1) | (2) | (2) | |
| Infiltration cellular, mononuclear cell | | | 1 (50%) | |
| Necrosis | | | 1 (50%) | |
| Genital System | | | | |
| Epididymis | (48) | (48) | (48) | (48) |
| Granuloma sperm | | | | 1 (2%) |
| Hemorrhage | | | | 1 (2%) |
| Preputial gland | (48) | (48) | (46) | (48) |
| Atrophy | | | 1 (2%) | |
| Ectasia | 2 (4%) | 2 (4%) | 1 (2%) | |
| Infiltration cellular, mononuclear cell | 6 (13%) | 5 (10%) | 7 (15%) | 3 (6%) |
| Inflammation, chronic | 5 (10%) | 5 (10%) | 4 (9%) | 1 (2%) |
| Inflammation, suppurative | 1 (2%) | 4 (8%) | 7 (15%) | 3 (6%) |
| Prostate | (46) | (46) | (44) | (44) |
| Hyperplasia | | 1 (2%) | | |
| Inflammation, chronic | | | 1 (2%) | |
| Inflammation, suppurative | 1 (2%) | | 1 (2%) | |
| Seminal vesicle | (48) | (47) | (47) | (44) |
| Dilatation | 4 (8%) | 1 (2%) | 2 (4%) | |
| Hyperplasia | 1 (2%) | | | |
| Inflammation, chronic | 2 (4%) | | 2 (4%) | |
| Inflammation, suppurative | 1 (2%) | 1 (2%) | | |
| Testes | (48) | (48) | (48) | (48) |
| Atrophy, focal | | | 1 (2%) | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|------------------------------------|----------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Hematopoietic System | | | | |
| Bone marrow | (48) | (48) | (47) | (47) |
| Hyperplasia, neutrophil | 2 (4%) | | 1 (2%) | 1 (2%) |
| Lymph node | (3) | (1) | (2) | |
| Iliac, hemorrhage | | | 1 (50%) | |
| Iliac, hyperplasia, lymphoid | 1 (33%) | | | |
| Iliac, hyperplasia, plasma cell | | | 2 (100%) | |
| Iliac, pigmentation, hemosiderin | | | 1 (50%) | |
| Renal, hyperplasia, lymphoid | 1 (33%) | | | |
| Lymph node, bronchial | (36) | (36) | (35) | (26) |
| Hemorrhage | | 2 (6%) | | |
| Hyperplasia, lymphoid | | 2 (6%) | 1 (3%) | |
| Pigmentation, hemosiderin | | 1 (3%) | | |
| Lymph node, mandibular | (36) | (34) | (32) | (34) |
| Congestion | | 1 (3%) | | |
| Hemorrhage | 1 (3%) | | | |
| Hyperplasia, histiocytic | | 1 (3%) | 2 (6%) | |
| Hyperplasia, lymphoid | | 1 (3%) | 1 (3%) | |
| Pigmentation, hemosiderin | | 2 (6%) | 1 (3%) | |
| Lymph node, mesenteric | (47) | (42) | (41) | (40) |
| Hematopoietic cell proliferation | | | 1 (2%) | 1 (3%) |
| Hemorrhage | 1 (2%) | 2 (5%) | 2 (5%) | |
| Hyperplasia, histiocytic | 2 (4%) | 1 (2%) | | |
| Hyperplasia, lymphoid | | 1 (2%) | 2 (5%) | |
| Inflammation, chronic active | | 1 (2%) | 1 (2%) | 1 (3%) |
| Lymph node, mediastinal | (30) | (34) | (32) | (29) |
| Congestion | | 1 (3%) | | |
| Hematopoietic cell proliferation | | | 1 (3%) | |
| Hemorrhage | | | 3 (9%) | 2 (7%) |
| Hyperplasia, histiocytic | | | 1 (3%) | 2 (7%) |
| Hyperplasia, lymphoid | 1 (3%) | | 1 (3%) | |
| Spleen | (48) | (48) | (46) | (46) |
| Atrophy | | 1 (2%) | | |
| Hematopoietic cell proliferation | 14 (29%) | 32 (67%) | 41 (89%) | 42 (91%) |
| Hyperplasia, lymphoid | | 1 (2%) | | |
| Pigmentation, melanin | | 2 (4%) | | |
| Thymus | (43) | (40) | (34) | (37) |
| Mineralization | | | 1 (3%) | |
| Integumentary System | | | | |
| Skin | (48) | (48) | (48) | (48) |
| Acanthosis | 1 (2%) | | | |
| Atrophy | | | 1 (2%) | |
| Inflammation, chronic | 2 (4%) | 4 (8%) | 2 (4%) | 3 (6%) |
| Inflammation, suppurative | | | | 1 (2%) |
| Ulcer | 2 (4%) | 4 (8%) | | 5 (10%) |
| Prepuce, acanthosis | 1 (2%) | 2 (4%) | | |
| Prepuce, inflammation, chronic | | 2 (4%) | | |
| Prepuce, inflammation, suppurative | | | | 1 (2%) |
| Prepuce, pigmentation, melanin | | 1 (2%) | | |
| Prepuce, ulcer | | 1 (2%) | 1 (2%) | |
| Subcutaneous tissue, congestion | | | | 1 (2%) |
| Subcutaneous tissue, hemorrhage | | | 1 (2%) | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Musculoskeletal System | | | | |
| Bone | (48) | (48) | (48) | (48) |
| Fracture | | | | 1 (2%) |
| Sternum, developmental malformation | | | | 2 (4%) |
| Nervous System | | | | |
| Brain | (48) | (48) | (48) | (48) |
| Hemorrhage | 1 (2%) | 1 (2%) | | |
| Mineralization | 23 (48%) | 19 (40%) | 13 (27%) | 24 (50%) |
| Necrosis, liquifactive | 2 (4%) | | | |
| Respiratory System | | | | |
| Larynx | (47) | (48) | (46) | (46) |
| Cytoplasmic alteration | 1 (2%) | | | |
| Exudate, mucopurulent | | | 2 (4%) | 1 (2%) |
| Hemorrhage | | | | 1 (2%) |
| Metaplasia, squamous | | | | 2 (4%) |
| Lung | (48) | (48) | (48) | (48) |
| Congestion | | 2 (4%) | 5 (10%) | 1 (2%) |
| Crystals | | 1 (2%) | | |
| Hemorrhage | 6 (13%) | 4 (8%) | 2 (4%) | 1 (2%) |
| Infarct | | | | 1 (2%) |
| Pigmentation, hemosiderin | | | 1 (2%) | |
| Alveolar epithelium, hyperplasia | 1 (2%) | | 1 (2%) | 1 (2%) |
| Alveolus, infiltration cellular, histiocyte | 4 (8%) | 5 (10%) | 1 (2%) | 1 (2%) |
| Interstitial, infiltration cellular, mixed cell | 1 (2%) | | | |
| Nose | (48) | (47) | (46) | (47) |
| Inflammation, suppurative | 1 (2%) | | 3 (7%) | 1 (2%) |
| Proliferation connective tissue, multifocal | | 1 (2%) | | |
| Nasolacrimal duct, inflammation, suppurative | | | | 1 (2%) |
| Respiratory epithelium, cytoplasmic alteration | 1 (2%) | | | |
| Respiratory epithelium, degeneration | | | | 1 (2%) |
| Vomeronasal organ, hypertrophy | | | 1 (2%) | |
| Trachea | (47) | (47) | (46) | (46) |
| Cytoplasmic alteration | 1 (2%) | | | |
| Special Senses System | | | | |
| Eye | | (1) | (2) | |
| Degeneration, diffuse | | | 1 (50%) | |
| Phthisis bulbi | | | 1 (50%) | |
| Retinal detachment | | | 1 (50%) | |
| Cornea, inflammation, chronic | | | 1 (50%) | |
| Harderian gland | (6) | (8) | (5) | (6) |
| Hyperplasia | | | 1 (20%) | 1 (17%) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Urinary System | | | | |
| Kidney | (48) | (48) | (48) | (48) |
| Cyst | 2 (4%) | | 1 (2%) | 1 (2%) |
| Hemorrhage | 1 (2%) | | | |
| Infiltration cellular, mononuclear cell | 2 (4%) | 1 (2%) | | 1 (2%) |
| Inflammation, chronic active | 1 (2%) | | 1 (2%) | |
| Inflammation, suppurative | 2 (4%) | | | |
| Nephropathy | 32 (67%) | 12 (25%) | 10 (21%) | 16 (33%) |
| Renal tubule, dilatation | | 4 (8%) | 16 (33%) | 36 (75%) |
| Renal tubule, karyomegaly | 1 (2%) | 2 (4%) | 10 (21%) | 28 (58%) |
| Urinary bladder | (48) | (43) | (43) | (42) |
| Infiltration cellular, mononuclear cell | 1 (2%) | | | |
| Inflammation, chronic active | 2 (4%) | | 1 (2%) | 1 (2%) |
| Inflammation, suppurative | 1 (2%) | 1 (2%) | | |

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

| | | |
|------------------|---|------------|
| TABLE D1 | Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene | 211 |
| TABLE D2 | Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene | 216 |
| TABLE D3 | Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene | 240 |
| TABLE D4a | Historical Incidence of Liver Neoplasms in Chamber Control Female B6C3F₁ Mice | 244 |
| TABLE D4b | Historical Incidence of Histiocytic Sarcoma in Chamber Control Female B6C3F₁ Mice | 245 |
| TABLE D5 | Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene | 246 |

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene ^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|---------|---------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 58 | 58 | 58 | 58 |
| 15-Month interim evaluation | 10 | 10 | 10 | 10 |
| Early deaths | | | | |
| Accidental death | | | 1 | |
| Moribund | 8 | 28 | 26 | 27 |
| Natural deaths | 4 | 16 | 15 | 17 |
| Survivors | | | | |
| Terminal sacrifice | 36 | 4 | 6 | 4 |
| Animals examined microscopically | 58 | 58 | 57 | 58 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Intestine small, jejunum | (10) | (10) | (10) | (10) |
| Liver | (10) | (10) | (10) | (10) |
| Hemangiosarcoma | | 1 (10%) | | |
| Hepatocellular carcinoma | | 3 (30%) | 1 (10%) | 3 (30%) |
| Hepatocellular adenoma | | 2 (20%) | 3 (30%) | 1 (10%) |
| Hepatocellular adenoma, multiple | | | | 1 (10%) |
| Endocrine System | | | | |
| Adrenal medulla | (10) | (10) | (10) | (10) |
| Pheochromocytoma benign | 1 (10%) | | | |
| Thyroid gland | (10) | (10) | (10) | (10) |
| Follicular cell, adenoma | 1 (10%) | | | |
| Respiratory System | | | | |
| Lung | (10) | (10) | (10) | (10) |
| Alveolar/bronchiolar adenoma | | | 1 (10%) | |
| Alveolar/bronchiolar adenoma, multiple | | 1 (10%) | | |
| Hepatocellular carcinoma, metastatic, liver | | | | 1 (10%) |
| Special Senses System | | | | |
| Harderian gland | (1) | | | (2) |
| Adenoma | 1 (100%) | | | 1 (50%) |
| Bilateral, adenoma | | | | 1 (50%) |
| Systemic Lesions | | | | |
| Multiple organs ^b | (10) | (10) | (10) | (10) |
| Lymphoma malignant mixed | | | | 1 (10%) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|----------|----------|----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Systems Examined With No Neoplasms Observed | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| Genital System | | | | |
| Hematopoietic System | | | | |
| Integumentary System | | | | |
| Musculoskeletal System | | | | |
| Nervous System | | | | |
| Urinary System | | | | |
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Intestine large, colon | (47) | (45) | (42) | (40) |
| Sarcoma | | 1 (2%) | | |
| Intestine large, cecum | (47) | (42) | (40) | (36) |
| Leiomyosarcoma | 1 (2%) | | | |
| Sarcoma | | 1 (2%) | | |
| Intestine small, duodenum | (45) | (39) | (39) | (35) |
| Sarcoma | | 1 (3%) | | |
| Intestine small, jejunum | (46) | (37) | (39) | (35) |
| Intestine small, ileum | (46) | (39) | (40) | (35) |
| Leiomyosarcoma, metastatic, uterus | | 1 (3%) | | |
| Sarcoma | | 1 (3%) | | |
| Liver | (48) | (48) | (47) | (47) |
| Hemangioma | | 4 (8%) | 1 (2%) | 1 (2%) |
| Hemangioma, multiple | | 1 (2%) | 1 (2%) | |
| Hemangiosarcoma | | 19 (40%) | 15 (32%) | 19 (40%) |
| Hemangiosarcoma, multiple | | 8 (17%) | 12 (26%) | 15 (32%) |
| Hepatocellular carcinoma | 4 (8%) | 23 (48%) | 15 (32%) | 13 (28%) |
| Hepatocellular carcinoma, multiple | | 5 (10%) | 7 (15%) | 7 (15%) |
| Hepatocellular adenoma | 14 (29%) | 10 (21%) | 11 (23%) | 8 (17%) |
| Hepatocellular adenoma, multiple | 1 (2%) | 7 (15%) | 9 (19%) | 7 (15%) |
| Histiocytic sarcoma | 1 (2%) | 21 (44%) | 19 (40%) | 18 (38%) |
| Sarcoma | | 1 (2%) | | |
| Mesentery | (9) | (13) | (7) | (6) |
| Hemangiosarcoma | | 2 (15%) | | |
| Histiocytic sarcoma | 1 (11%) | 1 (8%) | | |
| Leiomyosarcoma, metastatic, uterus | | 1 (8%) | | |
| Sarcoma | | 1 (8%) | | 1 (17%) |
| Pancreas | (48) | (47) | (44) | (47) |
| Hemangiosarcoma | | | | 1 (2%) |
| Histiocytic sarcoma | | 2 (4%) | 1 (2%) | 1 (2%) |
| Leiomyosarcoma, metastatic, uterus | | 1 (2%) | | |
| Sarcoma | | 1 (2%) | | |
| Stomach, forestomach | (48) | (48) | (47) | (47) |
| Leiomyosarcoma, metastatic, uterus | | 1 (2%) | | |
| Squamous cell papilloma | | 1 (2%) | 1 (2%) | |
| Stomach, glandular | (48) | (48) | (45) | (45) |
| Leiomyosarcoma, metastatic, uterus | | 1 (2%) | | |
| Sarcoma | | 1 (2%) | | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|---------|----------|---------|-----------|
| 2-Year Study (continued) | | | | |
| Cardiovascular System | | | | |
| Heart | (48) | (48) | (47) | (48) |
| Histiocytic sarcoma | | | 1 (2%) | 1 (2%) |
| Endocrine System | | | | |
| Adrenal cortex | (48) | (47) | (47) | (47) |
| Adenoma | | 1 (2%) | | |
| Histiocytic sarcoma | 1 (2%) | 3 (6%) | | 1 (2%) |
| Capsule, adenoma | 1 (2%) | | | |
| Capsule, carcinoma | 1 (2%) | | | |
| Islets, pancreatic | (48) | (45) | (44) | (47) |
| Adenoma | 1 (2%) | 1 (2%) | | |
| Pituitary gland | (46) | (45) | (44) | (46) |
| Histiocytic sarcoma | 1 (2%) | | | |
| Pars distalis, adenoma | 4 (9%) | 5 (11%) | 2 (5%) | 6 (13%) |
| Pars intermedia, adenoma | 1 (2%) | | | |
| Thyroid gland | (48) | (48) | (46) | (46) |
| Follicular cell, adenoma | 2 (4%) | 1 (2%) | 1 (2%) | 1 (2%) |
| General Body System | | | | |
| Tissue NOS | | (1) | | |
| Sarcoma | | 1 (100%) | | |
| Genital System | | | | |
| Ovary | (47) | (47) | (45) | (46) |
| Cystadenoma | 2 (4%) | | | 2 (4%) |
| Hemangiosarcoma | | 1 (2%) | | |
| Histiocytic sarcoma | 1 (2%) | 5 (11%) | 4 (9%) | 3 (7%) |
| Luteoma | 2 (4%) | | | |
| Sarcoma | | 1 (2%) | | |
| Periovarian tissue, histiocytic sarcoma | | | 1 (2%) | |
| Uterus | (48) | (48) | (45) | (47) |
| Histiocytic sarcoma | 1 (2%) | | | |
| Leiomyosarcoma | | 1 (2%) | | |
| Polyp stromal | 1 (2%) | | | 1 (2%) |
| Cervix, granular cell tumor NOS | 1 (2%) | | | |
| Hematopoietic System | | | | |
| Bone marrow | (48) | (48) | (46) | (47) |
| Histiocytic sarcoma | | 6 (13%) | 5 (11%) | 4 (9%) |
| Lymph node | (5) | (6) | (4) | (6) |
| Axillary, histiocytic sarcoma | 1 (20%) | | | |
| Iliac, histiocytic sarcoma | 1 (20%) | 1 (17%) | 1 (25%) | 1 (17%) |
| Pancreatic, histiocytic sarcoma | | | 1 (25%) | 1 (17%) |
| Renal, histiocytic sarcoma | | 1 (17%) | 2 (50%) | 2 (33%) |
| Lymph node, bronchial | (40) | (36) | (40) | (38) |
| Histiocytic sarcoma | | 4 (11%) | 6 (15%) | 4 (11%) |
| Sarcoma | | 1 (3%) | | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|---------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Hematopoietic System (continued) | | | | |
| Lymph node, mandibular | (39) | (35) | (34) | (32) |
| Histiocytic sarcoma | 1 (3%) | 3 (9%) | 2 (6%) | 2 (6%) |
| Lymph node, mesenteric | (43) | (40) | (41) | (43) |
| Hemangioma | | | 1 (2%) | |
| Histiocytic sarcoma | 1 (2%) | 6 (15%) | 7 (17%) | 6 (14%) |
| Lymph node, mediastinal | (28) | (35) | (35) | (33) |
| Histiocytic sarcoma | 1 (4%) | 9 (26%) | 9 (26%) | 4 (12%) |
| Spleen | (48) | (48) | (46) | (47) |
| Histiocytic sarcoma | 1 (2%) | 7 (15%) | 8 (17%) | 9 (19%) |
| Sarcoma | | 1 (2%) | | |
| Thymus | (46) | (42) | (39) | (38) |
| Histiocytic sarcoma | | 2 (5%) | 2 (5%) | 2 (5%) |
| Integumentary System | | | | |
| Mammary gland | (48) | (46) | (45) | (46) |
| Carcinoma | 1 (2%) | | | |
| Skin | (47) | (48) | (47) | (48) |
| Hemangioma | | | 1 (2%) | |
| Squamous cell papilloma | | | | 1 (2%) |
| Subcutaneous tissue, sarcoma | | | | 2 (4%) |
| Musculoskeletal System | | | | |
| Bone | (48) | (48) | (47) | (48) |
| Osteosarcoma | 1 (2%) | | | |
| Rib, leiomyosarcoma, metastatic, uterus | | 1 (2%) | | |
| Skeletal muscle | | (1) | | |
| Leiomyosarcoma, metastatic, uterus | | 1 (100%) | | |
| Nervous System | | | | |
| Brain | (48) | (48) | (47) | (48) |
| Astrocytoma NOS | 1 (2%) | | | |
| Histiocytic sarcoma | | 1 (2%) | | 1 (2%) |
| Respiratory System | | | | |
| Lung | (48) | (48) | (47) | (47) |
| Alveolar/bronchiolar adenoma | 5 (10%) | | 5 (11%) | 3 (6%) |
| Alveolar/bronchiolar adenoma, multiple | 1 (2%) | | 1 (2%) | |
| Alveolar/bronchiolar carcinoma | | | 2 (4%) | 1 (2%) |
| Carcinoma | | 1 (2%) | | |
| Carcinoma, metastatic, mammary gland | 1 (2%) | | | |
| Hemangiosarcoma, metastatic, liver | | | | 3 (6%) |
| Hemangiosarcoma, metastatic, pancreas | | | | 1 (2%) |
| Hepatocellular carcinoma, metastatic, liver | 1 (2%) | 8 (17%) | 6 (13%) | 4 (9%) |
| Histiocytic sarcoma | 1 (2%) | 16 (33%) | 13 (28%) | 13 (28%) |
| Osteosarcoma, metastatic, bone | 1 (2%) | | | |
| Sarcoma | | 1 (2%) | | |
| Sarcoma, metastatic, skin | | | | 1 (2%) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|---------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Special Senses System | | | | |
| Harderian gland | (3) | (6) | (4) | (6) |
| Adenoma | 2 (67%) | 3 (50%) | | 6 (100%) |
| Urinary System | | | | |
| Kidney | (48) | (48) | (47) | (47) |
| Histiocytic sarcoma | 1 (2%) | 7 (15%) | 4 (9%) | 3 (6%) |
| Osteosarcoma, metastatic, bone | 1 (2%) | | | |
| Urinary bladder | (46) | (43) | (43) | (37) |
| Leiomyosarcoma, metastatic, uterus | | 1 (2%) | | |
| Sarcoma | | 1 (2%) | | |
| Systemic Lesions | | | | |
| Multiple organs | (48) | (48) | (47) | (48) |
| Histiocytic sarcoma | 1 (2%) | 21 (44%) | 19 (40%) | 18 (38%) |
| Lymphoma malignant lymphocytic | | 1 (2%) | | |
| Lymphoma malignant mixed | 5 (10%) | 2 (4%) | | 2 (4%) |
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | | | | |
| 15-Month interim evaluation | 2 | 6 | 5 | 6 |
| 2-Year study | 34 | 47 | 45 | 46 |
| Total Primary Neoplasms | | | | |
| 15-Month interim evaluation | 3 | 7 | 5 | 8 |
| 2-Year study | 53 | 132 | 104 | 115 |
| Total animals with benign neoplasms | | | | |
| 15-Month interim evaluation | 2 | 3 | 4 | 4 |
| 2-Year study | 27 | 24 | 26 | 29 |
| Total benign neoplasms | | | | |
| 15-Month interim evaluation | 3 | 3 | 4 | 4 |
| 2-Year study | 37 | 34 | 34 | 36 |
| Total animals with malignant neoplasms | | | | |
| 15-Month interim evaluation | | 3 | 1 | 4 |
| 2-Year study | 14 | 46 | 45 | 46 |
| Total malignant neoplasms | | | | |
| 15-Month interim evaluation | | 4 | 1 | 4 |
| 2-Year study | 14 | 98 | 70 | 79 |
| Total animals with metastatic neoplasms | | | | |
| 15-Month interim evaluation | | | | 1 |
| 2-Year study | 3 | 9 | 6 | 9 |
| Total metastatic neoplasms | | | | |
| 15-Month interim evaluation | | | | 1 |
| 2-Year study | 4 | 16 | 6 | 9 |
| Total animals with uncertain neoplasms- benign or malignant | | | | |
| 2-Year study | 2 | | | |
| Total uncertain neoplasms | | | | |
| 2-Year study | 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 D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene: 312 ppm
 (continued)

| | |
|---|---|
| Number of Days on Study | 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 |
| | 2 2 5 5 9 3 3 3 4 4 4 4 6 8 8 9 0 0 0 0 0 1 1 1 1 |
| | 9 9 4 5 3 0 0 9 1 1 1 1 6 5 8 6 0 0 3 7 9 4 4 4 4 |
| Carcass ID Number | 1 2 1 2 1 1 2 1 1 1 2 2 2 2 2 2 1 1 1 2 2 1 1 2 2 |
| | 9 1 8 0 9 7 1 8 7 9 1 2 0 3 0 1 7 8 8 0 2 7 9 1 2 |
| | 2 2 0 5 6 6 6 7 7 4 9 3 3 1 7 3 9 2 8 6 0 5 7 1 5 |
| Endocrine System (continued) | |
| Parathyroid gland | M + + M + M + + + M M + M + + M M M + M M M + M + |
| Pituitary gland | + + + + + + + I + + + + + + + + + + + + + + + + |
| Pars distalis, adenoma | |
| Thyroid gland | + |
| Follicular cell, adenoma | |
| General Body System | |
| Tissue NOS | |
| Sarcoma | |
| Genital System | |
| Clitoral gland | + + M + + + + M + + + M + M + + + + + M + + + + M |
| Ovary | + + M + |
| Hemangiosarcoma | |
| Histiocytic sarcoma | X X X X |
| Sarcoma | |
| Uterus | + |
| Leiomyosarcoma | X |
| Hematopoietic System | |
| Bone marrow | + |
| Histiocytic sarcoma | X |
| Lymph node | |
| Iliac, histiocytic sarcoma | |
| Renal, histiocytic sarcoma | |
| Lymph node, bronchial | + + M M + + + M I + + + M + + + + M + + + + + M |
| Histiocytic sarcoma | X |
| Sarcoma | |
| Lymph node, mandibular | + + M + M + + + + + + + M + + + + M M M + + + + + |
| Histiocytic sarcoma | X |
| Lymph node, mesenteric | + + + + + I M + + + + + + + + M + + + + + + + + |
| Histiocytic sarcoma | X |
| Lymph node, mediastinal | + + M + + M M + + + + + + + + I M + + I M M + + + |
| Histiocytic sarcoma | X |
| Spleen | + |
| Histiocytic sarcoma | X |
| Sarcoma | |
| Thymus | + + M + + + + + + + + + + + I M M + + + + + + + + |
| Histiocytic sarcoma | X |
| Integumentary System | |
| Mammary gland | + + + + + + + + + + + + + + M + + + + + + + + I |
| Skin | + |
| Musculoskeletal System | |
| Bone | + |
| Rib, leiomyosarcoma, metastatic, uterus | X |
| Skeletal muscle | |
| Leiomyosarcoma, metastatic, uterus | X |

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene: 312 ppm
 (continued)

| | |
|---|---|
| Number of Days on Study | 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 |
| | 2 2 5 5 9 3 3 3 4 4 4 4 6 8 8 9 0 0 0 0 0 1 1 1 1 |
| | 9 9 4 5 3 0 0 9 1 1 1 1 6 5 8 6 0 0 3 7 9 4 4 4 4 |
| Carcass ID Number | 1 2 1 2 1 1 2 1 1 1 2 2 2 2 2 2 1 1 1 2 2 1 1 2 2 |
| | 9 1 8 0 9 7 1 8 7 9 1 2 0 3 0 1 7 8 8 0 2 7 9 1 2 |
| | 2 2 0 5 6 6 6 7 7 4 9 3 3 1 7 3 9 2 8 6 0 5 7 1 5 |
| Nervous System | |
| Brain | + |
| Histiocytic sarcoma | X |
| Respiratory System | |
| Larynx | + + + + + + + A + + + + + + + + + + + + + + + + + |
| Lung | + |
| Carcinoma | X |
| Hepatocellular carcinoma, metastatic, liver | X X X X X |
| Histiocytic sarcoma | X X X X X X X X |
| Sarcoma | X X X X X X X X |
| Nose | + |
| Trachea | + |
| Special Senses System | |
| Ear | + + |
| Harderian gland | + + |
| Adenoma | |
| Urinary System | |
| Kidney | + |
| Histiocytic sarcoma | X |
| Urinary bladder | + + + A + + + A + + + + + + + + A + + + + + A + + + |
| Leiomyosarcoma, metastatic, uterus | X |
| Sarcoma | |
| Systemic Lesions | |
| Multiple organs | + |
| Histiocytic sarcoma | X |
| Lymphoma malignant lymphocytic | X |
| Lymphoma malignant mixed | |

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene: 625 ppm
 (continued)

| | | |
|--------------------------------|---|----------|
| Number of Days on Study | 5 6 | |
| | 9 0 1 1 1 2 3 3 3 3 4 5 5 5 5 5 6 6 6 6 6 6 | |
| | 7 8 4 5 9 9 0 0 1 6 2 0 2 3 3 3 1 7 7 7 7 7 7 | |
| Carcass ID Number | 3 3 3 2 2 3 3 3 2 3 3 3 3 3 3 3 3 3 3 3 3 3 | Total |
| | 1 1 2 9 9 1 0 1 9 3 4 3 3 3 3 4 4 0 1 2 2 3 4 | Tissues/ |
| | 9 4 7 6 3 5 6 3 9 3 2 2 0 5 6 7 0 3 0 1 8 1 6 | Tumors |
| Special Senses System | | |
| Harderian gland | + + | 4 |
| Urinary System | | |
| Kidney | + | 47 |
| Histiocytic sarcoma | | 4 |
| Urinary bladder | + + + + I + + + + + + + + + + + + M + + + + | 43 |
| Systemic Lesions | | |
| Multiple organs | + | 47 |
| Histiocytic sarcoma | X X X X | 19 |

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene: 1,250 ppm
 (continued)

| | | |
|---------------------------------|---|-----------------------------|
| Number of Days on Study | 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 | |
| | 8 8 8 8 9 9 0 0 0 0 0 1 2 2 2 3 4 4 4 4 6 6 6 6 | |
| | 7 8 8 9 7 7 0 6 8 8 8 9 3 6 9 9 2 6 6 7 7 7 7 | |
| Carcass ID Number | 4 | Total Tissues/ Tumors |
| | 5 1 5 3 5 5 5 4 2 3 4 6 6 4 2 3 2 3 4 3 3 4 4 | |
| | 7 8 9 2 2 6 8 9 0 7 4 4 0 8 3 8 4 0 3 4 6 0 7 | |
| Genital System | | |
| Clitoral gland | + + + + + + M M + M M + + + + + + + M + + + + | 39 |
| Ovary | + | 46 |
| Cystadenoma | X | 2 |
| Histiocytic sarcoma | | 3 |
| Uterus | + | 47 |
| Polyp stromal | | 1 |
| Hematopoietic System | | |
| Blood | | 1 |
| Bone marrow | + | 47 |
| Histiocytic sarcoma | | 4 |
| Lymph node | + | 6 |
| Iliac, histiocytic sarcoma | X | 1 |
| Pancreatic, histiocytic sarcoma | | 1 |
| Renal, histiocytic sarcoma | | 2 |
| Lymph node, bronchial | + + + + + + M M + + M + + + + + + M + + + M + + | 38 |
| Histiocytic sarcoma | X | 4 |
| Lymph node, mandibular | M + + + + + + M M M + M + M M + + + + + + + + | 32 |
| Histiocytic sarcoma | | 2 |
| Lymph node, mesenteric | + + + + + + + M + + + + + + + + + + + + + + + + | 43 |
| Histiocytic sarcoma | X X | 6 |
| Lymph node, mediastinal | + + + M + + M + M + + + + + + + + + M M + + + | 33 |
| Histiocytic sarcoma | X | 4 |
| Spleen | + | 47 |
| Histiocytic sarcoma | | 9 |
| Thymus | M + + + + + + + + M + M + + M M + + M + M + + | 38 |
| Histiocytic sarcoma | | 2 |
| Integumentary System | | |
| Mammary gland | M + + + + + + + + M + + + + + + + + + + + + + + | 46 |
| Skin | + | 48 |
| Squamous cell papilloma | | 1 |
| Subcutaneous tissue, sarcoma | X | 2 |
| Musculoskeletal System | | |
| Bone | + | 48 |
| Nervous System | | |
| Brain | + | 48 |
| Histiocytic sarcoma | | 1 |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|-------------|-------------|----------------|-------------|
| Harderian Gland: Adenoma | | | | |
| Overall rate ^a | 2/48 (4%) | 3/48 (6%) | 0/47 (0%) | 6/48 (13%) |
| Adjusted rate ^b | 5.6% | 39.7% | 0.0% | 61.6% |
| Terminal rate ^c | 2/36 (6%) | 1/4 (25%) | 0/6 (0%) | 2/4 (50%) |
| First incidence (days) | 667 (T) | 639 | — ^e | 484 |
| Life table test ^d | P<0.001 | P=0.012 | P=0.669N | P<0.001 |
| Logistic regression test ^d | P=0.009 | P=0.109 | P=0.669N | P=0.040 |
| Cochran-Armitage test ^d | P=0.078 | | | |
| Fisher exact test ^d | | P=0.500 | P=0.253N | P=0.134 |
| Liver: Hemangioma | | | | |
| Overall rate | 0/48 (0%) | 5/48 (10%) | 2/47 (4%) | 1/47 (2%) |
| Adjusted rate | 0.0% | 37.1% | 13.0% | 25.0% |
| Terminal rate | 0/36 (0%) | 0/4 (0%) | 0/6 (0%) | 1/4 (25%) |
| First incidence (days) | — | 541 | 569 | 667 (T) |
| Life table test | P=0.145 | P<0.001 | P=0.084 | P=0.091 |
| Logistic regression test | P=0.523 | P=0.024 | P=0.224 | P=0.091 |
| Cochran-Armitage test | P=0.510N | | | |
| Fisher exact test | | P=0.028 | P=0.242 | P=0.495 |
| Liver: Hemangiosarcoma | | | | |
| Overall rate | 0/48 (0%) | 27/48 (56%) | 27/47 (57%) | 34/47 (72%) |
| Adjusted rate | 0.0% | 100.0% | 95.5% | 93.5% |
| Terminal rate | 0/36 (0%) | 4/4 (100%) | 5/6 (83%) | 2/4 (50%) |
| First incidence (days) | — | 530 | 481 | 468 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |
| Liver: Hemangioma or Hemangiosarcoma | | | | |
| Overall rate | 0/48 (0%) | 31/48 (65%) | 28/47 (60%) | 35/48 (73%) |
| Adjusted rate | 0.0% | 100.0% | 96.0% | 96.7% |
| Terminal rate | 0/36 (0%) | 4/4 (100%) | 5/6 (83%) | 3/4 (75%) |
| First incidence (days) | — | 530 | 481 | 468 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |
| Liver: Hepatocellular Adenoma | | | | |
| Overall rate | 15/48 (31%) | 17/48 (35%) | 20/47 (43%) | 15/47 (32%) |
| Adjusted rate | 37.2% | 89.1% | 93.2% | 76.8% |
| Terminal rate | 11/36 (31%) | 3/4 (75%) | 5/6 (83%) | 2/4 (50%) |
| First incidence (days) | 595 | 530 | 541 | 536 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.090 | P=0.138 | P=0.016 | P=0.198 |
| Cochran-Armitage test | P=0.515 | | | |
| Fisher exact test | | P=0.414 | P=0.176 | P=0.560 |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-------------|-------------|-------------|-------------|
| Liver: Hepatocellular Carcinoma | | | | |
| Overall rate | 4/48 (8%) | 28/48 (58%) | 22/47 (47%) | 20/47 (43%) |
| Adjusted rate | 10.8% | 95.0% | 87.7% | 72.5% |
| Terminal rate | 3/36 (8%) | 3/4 (75%) | 4/6 (67%) | 1/4 (25%) |
| First incidence (days) | 660 | 530 | 478 | 484 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.042 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P=0.012 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |
| Liver: Hepatocellular Adenoma or Carcinoma | | | | |
| Overall rate | 17/48 (35%) | 33/48 (69%) | 29/47 (62%) | 28/47 (60%) |
| Adjusted rate | 42.2% | 100.0% | 95.6% | 88.6% |
| Terminal rate | 13/36 (36%) | 4/4 (100%) | 5/6 (83%) | 2/4 (50%) |
| First incidence (days) | 595 | 530 | 478 | 484 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.045 | P<0.001 | P<0.001 | P=0.005 |
| Cochran-Armitage test | P=0.055 | | | |
| Fisher exact test | | P=0.001 | P=0.009 | P=0.015 |
| Lung: Alveolar/bronchiolar Adenoma | | | | |
| Overall rate | 6/48 (13%) | 0/48 (0%) | 6/47 (13%) | 3/47 (6%) |
| Adjusted rate | 16.7% | 0.0% | 40.6% | 30.2% |
| Terminal rate | 6/36 (17%) | 0/4 (0%) | 2/6 (33%) | 1/4 (25%) |
| First incidence (days) | 667 (T) | — | 510 | 509 |
| Life table test | P=0.056 | P=0.442N | P=0.026 | P=0.162 |
| Logistic regression test | P=0.563 | P=0.442N | P=0.505 | P=0.636N |
| Cochran-Armitage test | P=0.424N | | | |
| Fisher exact test | | P=0.013N | P=0.605 | P=0.254N |
| Lung: Alveolar/bronchiolar Adenoma or Carcinoma | | | | |
| Overall rate | 6/48 (13%) | 1/48 (2%) | 8/47 (17%) | 4/47 (9%) |
| Adjusted rate | 16.7% | 2.5% | 49.2% | 33.8% |
| Terminal rate | 6/36 (17%) | 0/4 (0%) | 2/6 (33%) | 1/4 (25%) |
| First incidence (days) | 667 (T) | 541 | 510 | 509 |
| Life table test | P=0.017 | P=0.694N | P=0.003 | P=0.064 |
| Logistic regression test | P=0.413 | P=0.148N | P=0.232 | P=0.575 |
| Cochran-Armitage test | P=0.565 | | | |
| Fisher exact test | | P=0.056N | P=0.370 | P=0.384N |
| Pituitary Gland (Pars Distalis): Adenoma | | | | |
| Overall rate | 4/46 (9%) | 5/45 (11%) | 2/44 (5%) | 6/46 (13%) |
| Adjusted rate | 10.6% | 55.3% | 18.2% | 53.0% |
| Terminal rate | 3/36 (8%) | 2/4 (50%) | 0/6 (0%) | 1/4 (25%) |
| First incidence (days) | 617 | 585 | 652 | 565 |
| Life table test | P=0.002 | P=0.018 | P=0.374 | P=0.002 |
| Logistic regression test | P=0.071 | P=0.282 | P=0.665 | P=0.123 |
| Cochran-Armitage test | P=0.352 | | | |
| Fisher exact test | | P=0.486 | P=0.360N | P=0.370 |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|------------|-------------|-------------|-------------|
| All Organs: Hemangioma | | | | |
| Overall rate | 0/48 (0%) | 5/48 (10%) | 4/47 (9%) | 1/48 (2%) |
| Adjusted rate | 0.0% | 37.1% | 33.5% | 25.0% |
| Terminal rate | 0/36 (0%) | 0/4 (0%) | 1/6 (17%) | 1/4 (25%) |
| First incidence (days) | — | 541 | 569 | 667 (T) |
| Life table test | P=0.054 | P<0.001 | P=0.002 | P=0.091 |
| Logistic regression test | P=0.353 | P=0.024 | P=0.018 | P=0.091 |
| Cochran-Armitage test | P=0.544N | | | |
| Fisher exact test | | P=0.028 | P=0.056 | P=0.500 |
| All Organs: Hemangiosarcoma | | | | |
| Overall rate | 0/48 (0%) | 27/48 (56%) | 27/47 (57%) | 34/48 (71%) |
| Adjusted rate | 0.0% | 100.0% | 95.5% | 93.5% |
| Terminal rate | 0/36 (0%) | 4/4 (100%) | 5/6 (83%) | 2/4 (50%) |
| First incidence (days) | — | 530 | 481 | 468 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |
| All Organs: Hemangioma or Hemangiosarcoma | | | | |
| Overall rate | 0/48 (0%) | 31/48 (65%) | 28/47 (60%) | 35/48 (73%) |
| Adjusted rate | 0.0% | 100.0% | 96.0% | 96.7% |
| Terminal rate | 0/36 (0%) | 4/4 (100%) | 5/6 (83%) | 3/4 (75%) |
| First incidence (days) | — | 530 | 481 | 468 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |
| All Organs: Histiocytic Sarcoma | | | | |
| Overall rate | 1/48 (2%) | 21/48 (44%) | 19/47 (40%) | 18/48 (38%) |
| Adjusted rate | 2.1% | 77.6% | 51.3% | 78.2% |
| Terminal rate | 0/36 (0%) | 1/4 (25%) | 0/6 (0%) | 1/4 (25%) |
| First incidence (days) | 400 | 429 | 457 | 484 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.015 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P=0.002 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |
| All Organs: Lymphocytic or Mixed Malignant Lymphoma | | | | |
| Overall rate | 5/48 (10%) | 3/48 (6%) | 0/47 (0%) | 2/48 (4%) |
| Adjusted rate | 12.8% | 18.0% | 0.0% | 30.0% |
| Terminal rate | 3/36 (8%) | 0/4 (0%) | 0/6 (0%) | 1/4 (25%) |
| First incidence (days) | 595 | 530 | — | 608 |
| Life table test | P=0.471 | P=0.348 | P=0.250N | P=0.377 |
| Logistic regression test | P=0.276N | P=0.419N | P=0.068N | P=0.551N |
| Cochran-Armitage test | P=0.115N | | | |
| Fisher exact test | | P=0.357N | P=0.030N | P=0.218N |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|-------------|-------------|-------------|-------------|
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 27/48 (56%) | 24/48 (50%) | 26/47 (55%) | 29/48 (60%) |
| Adjusted rate | 64.2% | 100.0% | 95.0% | 100.0% |
| Terminal rate | 21/36 (58%) | 4/4 (100%) | 5/6 (83%) | 4/4 (100%) |
| First incidence (days) | 595 | 530 | 510 | 484 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P=0.005 | P=0.319 | P=0.133 | P=0.016 |
| Cochran-Armitage test | P=0.290 | | | |
| Fisher exact test | | P=0.341N | P=0.546N | P=0.418 |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 14/48 (29%) | 46/48 (96%) | 45/47 (96%) | 46/48 (96%) |
| Adjusted rate | 32.3% | 100.0% | 100.0% | 100.0% |
| Terminal rate | 7/36 (19%) | 4/4 (100%) | 6/6 (100%) | 4/4 (100%) |
| First incidence (days) | 400 | 429 | 457 | 366 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P<0.001 | P<0.001 | P<0.001 |
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 34/48 (71%) | 47/48 (98%) | 45/47 (96%) | 46/48 (96%) |
| Adjusted rate | 77.2% | 100.0% | 100.0% | 100.0% |
| Terminal rate | 26/36 (72%) | 4/4 (100%) | 6/6 (100%) | 4/4 (100%) |
| First incidence (days) | 400 | 429 | 457 | 366 |
| Life table test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Logistic regression test | P<0.001 | P<0.001 | P=0.001 | P<0.001 |
| Cochran-Armitage test | P<0.001 | | | |
| Fisher exact test | | P<0.001 | P=0.001 | P<0.001 |

(T)Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and pituitary 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 D4a
Historical Incidence of Liver Neoplasms in Chamber Control Female B6C3F₁ Mice^a

| Study | Incidence in Controls | | | | |
|--|-----------------------|-----------------|------------------------|--------------------------|-------------------------------------|
| | Hemangioma | Hemangiosarcoma | Hepatocellular Adenoma | Hepatocellular Carcinoma | Hepatocellular Adenoma or Carcinoma |
| Historical Incidence at Battelle Pacific Northwest Laboratories | | | | | |
| 1,3-Butadiene | 0/49 | 1/49 | 11/49 | 4/49 | 15/49 |
| Acetonitrile | 0/49 | 0/49 | 4/49 | 7/49 | 9/49 |
| Allyl Glycidyl Ether | 0/50 | 0/50 | 1/50 | 5/50 | 6/50 |
| α -Chloroacetophenone | 0/50 | 0/50 | 4/50 | 8/50 | 12/50 |
| Epinephrine Hydrochloride | 0/50 | 1/50 | 2/50 | 1/50 | 3/50 |
| Ethyl Chloride | 0/49 | 0/49 | 0/49 | 3/49 | 3/49 |
| Hexachlorocyclopentadiene | 0/49 | 0/49 | 5/49 | 4/49 | 9/49 |
| <i>o</i> -Chlorobenzalmalononitrile (CS-2) | 0/50 | 0/50 | 4/50 | 7/50 | 11/50 |
| Ozone | 0/50 | 0/50 | 20/50 | 15/50 | 27/50 |
| Total | 0/446 | 2/446 (0.5%) | 51/446 (11.4%) | 54/446 (12.1%) | 95/446 (21.3%) |
| Standard deviation | | 0.9% | 12.5% | 8.1% | 14.8% |
| Range | | 0%-2% | 0%-40% | 2%-30% | 6%-54% |
| Overall Historical Incidence | | | | | |
| Total | 1/937 (0.1%) | 5/937 (0.5%) | 114/937 (12.2%) | 103/937 (11.0%) | 200/937 (21.3%) |
| Standard deviation | 0.5% | 1.0% | 9.7% | 6.7% | 11.9% |
| Range | 0%-2% | 0%-3% | 0%-40% | 0%-30% | 3%-54% |

^a Data as of 12 May 1995

TABLE D4b
Historical Incidence of Histiocytic Sarcoma in Chamber Control Female B6C3F₁ Mice^a

| Study | Incidence in Controls |
|--|-----------------------|
| Historical Incidence at Battelle Pacific Northwest Laboratories | |
| 1,3-Butadiene | 3/50 |
| Acetonitrile | 2/49 |
| Allyl Glycidyl Ether | 3/50 |
| α -Chloroacetophenone | 1/50 |
| Epinephrine Hydrochloride | 1/50 |
| Ethyl Chloride | 0/49 |
| Hexachlorocyclopentadiene | 4/49 |
| <i>o</i> -Chlorobenzalmalononitrile (CS-2) | 0/50 |
| Ozone | 0/50 |
| Total | 14/447 (3.1%) |
| Standard deviation | 3.0% |
| Range | 0%-8% |
| Overall Historical Incidence | |
| Total | 26/941 (2.8%) |
| Standard deviation | 3.1% |
| Range | 0%-10% |

^a Data as of 12 May 1995

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|-----------|---------|-----------|
| Disposition Summary | | | | |
| Animals initially in study | 58 | 58 | 58 | 58 |
| 15-Month interim evaluation | | | | |
| Early deaths | | | | |
| Accidental death | | | 1 | |
| Moribund | 8 | 28 | 26 | 27 |
| Natural deaths | 4 | 16 | 15 | 17 |
| Survivors | | | | |
| Terminal sacrifice | 36 | 4 | 6 | 4 |
| Animals examined microscopically | 58 | 58 | 57 | 58 |
| 15-Month Interim Evaluation | | | | |
| Alimentary System | | | | |
| Intestine large, cecum | (10) | (10) | (10) | (10) |
| Developmental malformation | | | 1 (10%) | |
| Liver | (10) | (10) | (10) | (10) |
| Angiectasis | | 4 (40%) | 2 (20%) | 1 (10%) |
| Basophilic focus | | | | 1 (10%) |
| Clear cell focus | | | | 1 (10%) |
| Eosinophilic focus | | 1 (10%) | 4 (40%) | 5 (50%) |
| Fatty change | | | 1 (10%) | 1 (10%) |
| Hematopoietic cell proliferation | | | | 1 (10%) |
| Infarct, chronic | | | | 1 (10%) |
| Infiltration cellular, mixed cell | 1 (10%) | 4 (40%) | 3 (30%) | 1 (10%) |
| Mixed cell focus | | 1 (10%) | | 1 (10%) |
| Necrosis, coagulative, multifocal | | 1 (10%) | | |
| Thrombosis | | 1 (10%) | | |
| Hepatocyte, karyomegaly | | | | 1 (10%) |
| Mesentery | (1) | (1) | | (1) |
| Fat, necrosis | 1 (100%) | 1 (100%) | | 1 (100%) |
| Salivary glands | (10) | (10) | (10) | (10) |
| Infiltration cellular, mononuclear cell | 4 (40%) | 3 (30%) | 4 (40%) | 1 (10%) |
| Endocrine System | | | | |
| Adrenal cortex | (10) | (10) | (10) | (10) |
| Capsule, hyperplasia | 5 (50%) | 10 (100%) | 5 (50%) | 9 (90%) |
| X-zone, vacuolization cytoplasmic | 1 (10%) | 2 (20%) | 3 (30%) | 1 (10%) |
| Thyroid gland | (10) | (10) | (10) | (10) |
| Ultimobranchial cyst | | | 1 (10%) | |
| Genital System | | | | |
| Clitoral gland | (9) | (9) | (9) | (9) |
| Ectasia | | 1 (11%) | | |
| Infiltration cellular, mononuclear cell | 1 (11%) | | 1 (11%) | |
| Ovary | (10) | (10) | (10) | (10) |
| Abscess | | 1 (10%) | | |
| Cyst | 1 (10%) | 1 (10%) | | 2 (20%) |
| Uterus | (10) | (10) | (10) | (10) |
| Endometrium, hyperplasia, cystic | 4 (40%) | 6 (60%) | 4 (40%) | 7 (70%) |

^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 Tetrafluoroethylene (continued)

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|---------|---------|-----------|-----------|
| 15-Month Interim Evaluation (continued) | | | | |
| Hematopoietic System | | | | |
| Bone marrow | (10) | (10) | (10) | (10) |
| Hyperplasia, neutrophil | | 1 (10%) | 1 (10%) | 1 (10%) |
| Lymph node | | | (1) | |
| Pancreatic, hyperplasia, lymphoid | | | 1 (100%) | |
| Lymph node, mandibular | (7) | (4) | (9) | (6) |
| Hyperplasia, lymphoid | | 1 (25%) | | |
| Lymph node, mediastinal | (7) | (7) | (8) | (3) |
| Hemorrhage | | | 1 (13%) | |
| Hyperplasia, lymphoid | | | 1 (13%) | |
| Spleen | (10) | (10) | (10) | (10) |
| Hematopoietic cell proliferation | 2 (20%) | 4 (40%) | 4 (40%) | 3 (30%) |
| Hyperplasia, lymphoid | | | 1 (10%) | |
| Infiltration cellular, polymorphonuclear | | | | 1 (10%) |
| Nervous System | | | | |
| Brain | (10) | (10) | (9) | (10) |
| Mineralization | 4 (40%) | 1 (10%) | 1 (11%) | 4 (40%) |
| Respiratory System | | | | |
| Lung | (10) | (10) | (10) | (10) |
| Embolus, focal | | 1 (10%) | | |
| Hemorrhage | 1 (10%) | | 1 (10%) | |
| Urinary System | | | | |
| Kidney | (10) | (10) | (10) | (10) |
| Infiltration cellular, mononuclear cell | 2 (20%) | 2 (20%) | | 1 (10%) |
| Pelvis, infiltration cellular, mononuclear cell | | | 1 (10%) | |
| Renal tubule, dilatation | 1 (10%) | | | |
| Renal tubule, karyomegaly | | | 10 (100%) | 10 (100%) |
| Urinary bladder | (9) | (10) | (10) | (9) |
| Hyperplasia, lymphoid | 2 (22%) | 1 (10%) | | 1 (11%) |
| Systems Examined With No Lesions Observed | | | | |
| Cardiovascular System | | | | |
| General Body System | | | | |
| Integumentary System | | | | |
| Musculoskeletal System | | | | |
| Special Senses System | | | | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|---------|----------|----------|-----------|
| 2-Year Study | | | | |
| Alimentary System | | | | |
| Gallbladder | (42) | (36) | (34) | (34) |
| Inflammation, chronic active | 1 (2%) | | | |
| Intestine large, colon | (47) | (45) | (42) | (40) |
| Infarct | 1 (2%) | | | |
| Mesothelium, inflammation, chronic active | 2 (4%) | | | |
| Intestine large, rectum | (48) | (48) | (42) | (41) |
| Mesothelium, inflammation, chronic active | 1 (2%) | | | |
| Intestine large, cecum | (47) | (42) | (40) | (36) |
| Lymphoid tissue, hyperplasia, lymphoid | | 1 (2%) | | |
| Mesothelium, inflammation, chronic active | 1 (2%) | | | |
| Intestine small, duodenum | (45) | (39) | (39) | (35) |
| Mesothelium, inflammation, chronic active | 1 (2%) | | | |
| Peyer's patch, hyperplasia, lymphoid | 1 (2%) | | 1 (3%) | |
| Intestine small, jejunum | (46) | (37) | (39) | (35) |
| Hemorrhage | | 1 (3%) | | |
| Perforation | 1 (2%) | | | |
| Mesothelium, inflammation, chronic active | 1 (2%) | | | |
| Peyer's patch, hyperplasia, lymphoid | | | 1 (3%) | |
| Intestine small, ileum | (46) | (39) | (40) | (35) |
| Mesothelium, inflammation, chronic active | 2 (4%) | | | |
| Peyer's patch, hyperplasia, lymphoid | | 1 (3%) | 1 (3%) | |
| Liver | (48) | (48) | (47) | (47) |
| Angiectasis | 1 (2%) | 9 (19%) | 6 (13%) | 4 (9%) |
| Basophilic focus | | | 1 (2%) | 2 (4%) |
| Clear cell focus | 1 (2%) | | | |
| Eosinophilic focus | 5 (10%) | 13 (27%) | 12 (26%) | 7 (15%) |
| Fatty change | | 3 (6%) | | |
| Hematopoietic cell proliferation | 3 (6%) | 19 (40%) | 13 (28%) | 15 (32%) |
| Hyperplasia, lymphoid | | | 1 (2%) | |
| Infarct | 2 (4%) | | | 1 (2%) |
| Infiltration cellular, mixed cell | 8 (17%) | 8 (17%) | 9 (19%) | 3 (6%) |
| Inflammation, suppurative | 1 (2%) | | | |
| Mixed cell focus | 3 (6%) | | | 1 (2%) |
| Necrosis, coagulative, multifocal | 4 (8%) | 11 (23%) | 8 (17%) | 8 (17%) |
| Thrombosis | | | | 1 (2%) |
| Centrilobular, necrosis | | 3 (6%) | 1 (2%) | |
| Serosa, fibrosis | 1 (2%) | 1 (2%) | | |
| Mesentery | (9) | (13) | (7) | (6) |
| Angiectasis | 1 (11%) | | | |
| Cyst | | 1 (8%) | | |
| Hyperplasia, lymphoid | | | 1 (14%) | |
| Mineralization | | | 1 (14%) | |
| Fat, hemorrhage | | 1 (8%) | | |
| Fat, necrosis | 7 (78%) | 8 (62%) | 6 (86%) | 5 (83%) |
| Mesothelium, inflammation, chronic active | 1 (11%) | | | |
| Pancreas | (48) | (47) | (44) | (47) |
| Fibrosis | 1 (2%) | | 1 (2%) | |
| Infiltration cellular, lymphocyte | 1 (2%) | | | |
| Inflammation, acute | | | 1 (2%) | |
| Inflammation, chronic active | 3 (6%) | | | |
| Acinus, atrophy | 1 (2%) | 1 (2%) | 3 (7%) | |
| Acinus, vacuolization cytoplasmic | | | | 1 (2%) |
| Duct, ectasia | | | 1 (2%) | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|----------|----------|-----------|-----------|
| 2-Year Study (continued) | | | | |
| Alimentary System (continued) | | | | |
| Salivary glands | (48) | (48) | (47) | (48) |
| Infiltration cellular, mononuclear cell | 19 (40%) | 6 (13%) | 9 (19%) | 2 (4%) |
| Inflammation, focal, suppurative | | | | 1 (2%) |
| Stomach, forestomach | (48) | (48) | (47) | (47) |
| Hyperplasia, squamous | | 1 (2%) | | |
| Ulcer | 1 (2%) | | | |
| Epithelium, hyperplasia | 4 (8%) | | | 4 (9%) |
| Serosa, inflammation, chronic active | 1 (2%) | | | |
| Stomach, glandular | (48) | (48) | (45) | (45) |
| Mineralization | | 1 (2%) | | |
| Serosa, inflammation, chronic active | 1 (2%) | | | |
| Tooth | (2) | | | (3) |
| Dysplasia | 1 (50%) | | | 2 (67%) |
| Peridontal tissue, inflammation, suppurative | | | | 1 (33%) |
| Cardiovascular System | | | | |
| Heart | (48) | (48) | (47) | (48) |
| Cardiomyopathy | 2 (4%) | | | |
| Polyarteritis | | | 1 (2%) | |
| Endocrine System | | | | |
| Adrenal cortex | (48) | (47) | (47) | (47) |
| Cyst | | 1 (2%) | 1 (2%) | |
| Degeneration, fatty | | | | 1 (2%) |
| Hyperplasia | 1 (2%) | | | 1 (2%) |
| Capsule, hyperplasia | 45 (94%) | 45 (96%) | 47 (100%) | 41 (87%) |
| Capsule, mineralization | | 1 (2%) | | |
| X-zone, vacuolization cytoplasmic | 1 (2%) | | 1 (2%) | 2 (4%) |
| Adrenal medulla | (48) | (47) | (47) | (46) |
| Angiectasis | | | 1 (2%) | |
| Islets, pancreatic | (48) | (45) | (44) | (47) |
| Hyperplasia | | 1 (2%) | | |
| Pituitary gland | (46) | (45) | (44) | (46) |
| Pars distalis, angiectasis | | | | 2 (4%) |
| Pars distalis, congestion | 2 (4%) | | | 2 (4%) |
| Pars distalis, cyst | 3 (7%) | | | 1 (2%) |
| Pars distalis, hyperplasia | 6 (13%) | 3 (7%) | 2 (5%) | 5 (11%) |
| Thyroid gland | (48) | (48) | (46) | (46) |
| Inflammation, focal | 1 (2%) | 1 (2%) | | |
| Polyarteritis | | | 1 (2%) | |
| Ultimobranchial cyst | 2 (4%) | 2 (4%) | 2 (4%) | 2 (4%) |
| Follicle, cyst | 2 (4%) | 2 (4%) | | |
| Follicular cell, hyperplasia | 2 (4%) | | | |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| General Body System | | | | |
| None | | | | |
| Genital System | | | | |
| Clitoral gland | (42) | (42) | (38) | (39) |
| Infiltration cellular, mononuclear cell | 1 (2%) | 1 (2%) | | |
| Ovary | (47) | (47) | (45) | (46) |
| Atrophy | 2 (4%) | 4 (9%) | 5 (11%) | 2 (4%) |
| Congestion | | 1 (2%) | 2 (4%) | 1 (2%) |
| Cyst | 13 (28%) | 27 (57%) | 16 (36%) | 18 (39%) |
| Hemorrhage | | | 1 (2%) | |
| Inflammation, chronic | 1 (2%) | | | |
| Metaplasia, osseous | 1 (2%) | | | |
| Pigmentation, hemosiderin | | | 1 (2%) | |
| Pigmentation, hemosiderin, cholesterol | 1 (2%) | | | |
| Pigmentation, lipofuscin | | 1 (2%) | | |
| Polyarteritis | 1 (2%) | | | |
| Uterus | (48) | (48) | (45) | (47) |
| Dilatation | 3 (6%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Hemorrhage | | | | 1 (2%) |
| Inflammation, chronic active | 1 (2%) | | 1 (2%) | |
| Inflammation, suppurative | 1 (2%) | 1 (2%) | | |
| Necrosis | | | 1 (2%) | |
| Endometrium, hyperplasia, cystic | 23 (48%) | 21 (44%) | 24 (53%) | 24 (51%) |
| Hematopoietic System | | | | |
| Bone marrow | (48) | (48) | (46) | (47) |
| Hyperplasia, erythrocyte | | 2 (4%) | 2 (4%) | |
| Hyperplasia, neutrophil | 2 (4%) | | | 1 (2%) |
| Lymph node | (5) | (6) | (4) | (6) |
| Axillary, hyperplasia, lymphoid | | | 1 (25%) | |
| Iliac, angiectasis | | 1 (17%) | | |
| Iliac, hyperplasia, lymphoid | | 1 (17%) | 1 (25%) | |
| Iliac, inflammation, chronic active | | 1 (17%) | | |
| Lumbar, hyperplasia, lymphoid | | | 1 (25%) | |
| Pancreatic, hyperplasia, histiocytic | 1 (20%) | | | |
| Pancreatic, hyperplasia, lymphoid | | 1 (17%) | | 1 (17%) |
| Renal, hemorrhage | | 1 (17%) | | 2 (33%) |
| Renal, hyperplasia, lymphoid | | 1 (17%) | | |
| Lymph node, bronchial | (40) | (36) | (40) | (38) |
| Congestion | | | 1 (3%) | |
| Hemorrhage | | 1 (3%) | 2 (5%) | 3 (8%) |
| Hyperplasia, histiocytic | | 1 (3%) | 1 (3%) | |
| Hyperplasia, lymphoid | | 5 (14%) | 3 (8%) | |
| Hyperplasia, plasma cell | 1 (3%) | | 1 (3%) | |
| Inflammation | 1 (3%) | | | |
| Pigmentation, hemosiderin | 1 (3%) | | 1 (3%) | 1 (3%) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|---|----------|----------|----------|-----------|
| 2-Year Study (continued) | | | | |
| Hematopoietic System (continued) | | | | |
| Lymph node, mandibular | (39) | (35) | (34) | (32) |
| Hemorrhage | | 1 (3%) | 1 (3%) | |
| Hyperplasia, histiocytic | | | 1 (3%) | 1 (3%) |
| Hyperplasia, lymphoid | 2 (5%) | 1 (3%) | 2 (6%) | 1 (3%) |
| Hyperplasia, plasma cell | | 1 (3%) | | 1 (3%) |
| Inflammation | 1 (3%) | | | |
| Lymph node, mesenteric | (43) | (40) | (41) | (43) |
| Angiectasis | | | | 1 (2%) |
| Hemorrhage | 2 (5%) | 1 (3%) | 3 (7%) | 2 (5%) |
| Hyperplasia, histiocytic | 1 (2%) | 1 (3%) | 3 (7%) | |
| Hyperplasia, lymphoid | | 5 (13%) | 4 (10%) | 2 (5%) |
| Hyperplasia, plasma cell | 1 (2%) | | | |
| Inflammation, chronic active | | 2 (5%) | | |
| Lymph node, mediastinal | (28) | (35) | (35) | (33) |
| Congestion | | | 1 (3%) | 2 (6%) |
| Hematopoietic cell proliferation | | | | 1 (3%) |
| Hemorrhage | | | 3 (9%) | |
| Hyperplasia, histiocytic | | 2 (6%) | | |
| Hyperplasia, lymphoid | 2 (7%) | 2 (6%) | 4 (11%) | |
| Hyperplasia, plasma cell | 1 (4%) | | | |
| Inflammation | 1 (4%) | | | |
| Pigmentation, hemosiderin | | 1 (3%) | 1 (3%) | |
| Spleen | (48) | (48) | (46) | (47) |
| Hematopoietic cell proliferation | 18 (38%) | 39 (81%) | 41 (89%) | 41 (87%) |
| Hyperplasia, lymphoid | 2 (4%) | | 2 (4%) | 1 (2%) |
| Pigmentation, melanin | | 1 (2%) | | |
| Capsule, hematocyst | | | 1 (2%) | |
| Thymus | (46) | (42) | (39) | (38) |
| Atrophy | | | 1 (3%) | |
| Hyperplasia, lymphoid | 1 (2%) | | | |
| Necrosis | | | 4 (10%) | |
| Integumentary System | | | | |
| Mammary gland | (48) | (46) | (45) | (46) |
| Hyperplasia | | 1 (2%) | 2 (4%) | 2 (4%) |
| Skin | (47) | (48) | (47) | (48) |
| Congestion | | 1 (2%) | | |
| Musculoskeletal System | | | | |
| Bone | (48) | (48) | (47) | (48) |
| Fracture healed | | 1 (2%) | | |
| Nervous System | | | | |
| Brain | (48) | (48) | (47) | (48) |
| Compression | 1 (2%) | 1 (2%) | | 1 (2%) |
| Hemorrhage | | 1 (2%) | | |
| Mineralization | 20 (42%) | 9 (19%) | 14 (30%) | 21 (44%) |
| Ventricle, dilatation | | | | 1 (2%) |

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

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|--------|---------|---------|-----------|
| 2-Year Study (continued) | | | | |
| Respiratory System | | | | |
| Lung | (48) | (48) | (47) | (47) |
| Congestion | 2 (4%) | | 1 (2%) | |
| Hemorrhage | 2 (4%) | 5 (10%) | 5 (11%) | 3 (6%) |
| Inflammation, chronic | | | 2 (4%) | |
| Pigmentation, hemosiderin | | 2 (4%) | | 3 (6%) |
| Pigmentation, hemoglobin | | | 1 (2%) | |
| Alveolar epithelium, hyperplasia | | 1 (2%) | | 2 (4%) |
| Alveolus, infiltration cellular, histiocyte | 1 (2%) | 1 (2%) | 2 (4%) | 3 (6%) |
| Interstitialium, infiltration cellular, mixed cell | | 3 (6%) | 1 (2%) | |
| Nose | (47) | (48) | (45) | (45) |
| Inflammation, suppurative | | | | 1 (2%) |
| Nasolacrimal duct, inflammation, suppurative | | | | 1 (2%) |
| Respiratory epithelium, cytoplasmic alteration | | 1 (2%) | | |
| Special Senses System | | | | |
| Harderian gland | (3) | (6) | (4) | (6) |
| Hyperplasia | | 2 (33%) | 3 (75%) | |
| Urinary System | | | | |
| Kidney | (48) | (48) | (47) | (47) |
| Hemorrhage | 1 (2%) | | | |
| Infarct, chronic | | 2 (4%) | 1 (2%) | |
| Infiltration cellular, mononuclear cell | 3 (6%) | 1 (2%) | 4 (9%) | |
| Inflammation, chronic active | | | 1 (2%) | |
| Metaplasia, osseous | | | 1 (2%) | 1 (2%) |
| Nephropathy | | 1 (2%) | 1 (2%) | |
| Pigmentation, hemosiderin | | 1 (2%) | | 1 (2%) |
| Polyarteritis | | | 1 (2%) | |
| Renal tubule, karyomegaly | | | | 38 (81%) |
| Glomerulus, amyloid deposition | | | | 1 (2%) |
| Urinary bladder | (46) | (43) | (43) | (37) |
| Hyperplasia, lymphoid | 2 (4%) | 1 (2%) | 2 (5%) | |
| Infiltration cellular, mononuclear cell | 1 (2%) | | | |
| Inflammation, chronic active | | | 1 (2%) | |
| Metaplasia, squamous | | | 1 (2%) | |

APPENDIX E

GENETIC TOXICOLOGY

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL 254

RESULTS 254

TABLE E1 **Frequency of Micronuclei in Mouse Peripheral Blood Erythrocytes**
Following Treatment with Tetrafluoroethylene by Inhalation for 13 Weeks 254

GENETIC TOXICOLOGY

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented in MacGregor *et al.* (1990). Peripheral blood samples were obtained from male and female B6C3F₁ mice at the end of the 13-week study. Smears were immediately prepared and fixed in absolute methanol and stained with Giemsa. Slides were scanned at 630× or 1,000× magnification using a semi-automated image analysis system to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes (NCEs) in each of 10 animals per exposure group. The criteria of Schmid (1976) were used to define micronuclei; the minimum size limit was approximately 1/20 the diameter of the NCE.

Log transformation of the NCE data, testing for normality by the Shapiro-Wilk test, and testing for heterogeneity of variance by Cochran's test were performed before statistical analyses. The frequency of micronucleated cells among NCEs was analyzed by analysis of variance using the SAS GLM procedure. The NCE data for each exposure group were compared with the concurrent solvent control by Student's *t*-test.

RESULTS

No increases in the frequency of micronucleated NCEs were observed in peripheral blood samples obtained from male and female mice at the end of the 13-week inhalation study of tetrafluoroethylene (Table E1).

TABLE E1
Frequency of Micronuclei in Mouse Peripheral Blood Erythrocytes Following Treatment with Tetrafluoroethylene by Inhalation for 13 Weeks^a

| Dose (ppm) | Micronucleated Normochromatic Cells/ 1,000 Normochromatic Cells |
|---------------|--|
| Male | |
| 0 | 1.15 ± 0.10 |
| 1,250 | 1.31 ± 0.07 |
| 2,500 | 1.29 ± 0.07 |
| 5,000 | 1.29 ± 0.06 |
| | P=0.1276 ^b |
| Female | |
| 0 | 1.05 ± 0.08 |
| 1,250 | 1.04 ± 0.07 |
| 2,500 | 1.07 ± 0.07 |
| 5,000 | 1.14 ± 0.04 |
| | P=0.7150 |

^a Study was performed at USDA Western Regional Center. Data are presented as mean ± standard error. 10,000 normochromatic erythrocytes were scored for each of 10 animals per exposure group.

^b Trend test, linear contrasts from analysis of variance

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

| | | |
|-----------------|---|------------|
| TABLE F1 | Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Inhalation Study of Tetrafluoroethylene | 256 |
| TABLE F2 | Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Inhalation Study of Tetrafluoroethylene | 257 |
| TABLE F3 | Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluation in the 2-Year Inhalation Study of Tetrafluoroethylene | 258 |
| TABLE F4 | Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Inhalation Study of Tetrafluoroethylene | 259 |
| TABLE F5 | Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Inhalation Study of Tetrafluoroethylene | 260 |
| TABLE F6 | Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 15-Month Interim Evaluation in the 2-Year Inhalation Study of Tetrafluoroethylene | 261 |

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Inhalation Study
of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|-------------------|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| n | 5 | 5 | 5 | 5 | 5 | 5 |
| Male | | | | | | |
| Necropsy body wt | 153 ± 4 | 161 ± 4 | 160 ± 3 | 153 ± 1 | 148 ± 2 | 130 ± 3** |
| Brain | | | | | | |
| Absolute | 1.702 ± 0.013 | 1.752 ± 0.026 | 1.738 ± 0.025 | 1.724 ± 0.012 | 1.686 ± 0.021 | 1.648 ± 0.023 |
| Relative | 11.12 ± 0.21 | 10.93 ± 0.18 | 10.87 ± 0.31 | 11.27 ± 0.08 | 11.40 ± 0.24 | 12.72 ± 0.31** |
| Heart | | | | | | |
| Absolute | 0.522 ± 0.010 | 0.610 ± 0.015* | 0.602 ± 0.024* | 0.604 ± 0.023* | 0.594 ± 0.025 | 0.524 ± 0.014 |
| Relative | 3.41 ± 0.08 | 3.80 ± 0.07 | 3.77 ± 0.18 | 3.95 ± 0.17** | 4.01 ± 0.14** | 4.04 ± 0.10** |
| L. and R. Kidneys | | | | | | |
| Absolute | 1.200 ± 0.042 | 1.396 ± 0.037** | 1.402 ± 0.031** | 1.418 ± 0.017** | 1.512 ± 0.038** | 1.624 ± 0.049** |
| Relative | 7.82 ± 0.15 | 8.70 ± 0.08** | 8.75 ± 0.15** | 9.27 ± 0.15** | 10.21 ± 0.17** | 12.51 ± 0.27** |
| Liver | | | | | | |
| Absolute | 4.952 ± 0.289 | 6.094 ± 0.339 | 6.198 ± 0.411* | 5.888 ± 0.274 | 6.622 ± 0.257** | 5.282 ± 0.219 |
| Relative | 32.20 ± 1.24 | 38.02 ± 2.08* | 38.57 ± 1.92* | 38.50 ± 1.87* | 44.72 ± 1.60** | 40.71 ± 1.49** |
| Lung | | | | | | |
| Absolute | 0.886 ± 0.025 | 1.150 ± 0.103* | 1.288 ± 0.086** | 1.114 ± 0.033 | 0.978 ± 0.051 | 0.926 ± 0.016 |
| Relative | 5.79 ± 0.17 | 7.13 ± 0.51* | 8.03 ± 0.45** | 7.29 ± 0.24* | 6.60 ± 0.32 | 7.15 ± 0.20* |
| R. Testis | | | | | | |
| Absolute | 1.940 ± 0.039 | 2.070 ± 0.027 | 2.055 ± 0.054 | 2.018 ± 0.027 | 2.006 ± 0.023 | 1.728 ± 0.063** |
| Relative | 12.68 ± 0.43 | 12.93 ± 0.35 | 12.85 ± 0.44 | 13.19 ± 0.16 | 13.57 ± 0.32 | 13.33 ± 0.48 |
| Thymus | | | | | | |
| Absolute | 0.312 ± 0.017 | 0.355 ± 0.013 | 0.342 ± 0.019 | 0.313 ± 0.022 | 0.341 ± 0.012 | 0.258 ± 0.019 |
| Relative | 2.04 ± 0.12 | 2.21 ± 0.06 | 2.13 ± 0.08 | 2.05 ± 0.15 | 2.30 ± 0.06 | 1.98 ± 0.12 |
| Female | | | | | | |
| Necropsy body wt | 116 ± 2 | 121 ± 2 | 115 ± 3 | 120 ± 2 | 110 ± 3 | 101 ± 2** |
| Brain | | | | | | |
| Absolute | 1.656 ± 0.018 | 1.686 ± 0.036 | 1.648 ± 0.021 | 1.620 ± 0.030 | 1.620 ± 0.007 | 1.620 ± 0.039 |
| Relative | 14.34 ± 0.31 | 13.95 ± 0.21 | 14.32 ± 0.23 | 13.57 ± 0.41 | 14.84 ± 0.49 | 16.16 ± 0.59* |
| Heart | | | | | | |
| Absolute | 0.458 ± 0.008 | 0.498 ± 0.010* | 0.458 ± 0.013 | 0.470 ± 0.014 | 0.464 ± 0.005 | 0.438 ± 0.006 |
| Relative | 3.97 ± 0.11 | 4.12 ± 0.07 | 3.98 ± 0.11 | 3.93 ± 0.09 | 4.25 ± 0.13 | 4.36 ± 0.08* |
| L. and R. Kidneys | | | | | | |
| Absolute | 1.008 ± 0.020 | 1.130 ± 0.021 | 1.082 ± 0.020 | 1.142 ± 0.040* | 1.202 ± 0.045** | 1.456 ± 0.061** |
| Relative | 8.73 ± 0.25 | 9.35 ± 0.04 | 9.41 ± 0.27 | 9.54 ± 0.23 | 10.97 ± 0.26** | 14.49 ± 0.51** |
| Liver | | | | | | |
| Absolute | 4.016 ± 0.204 | 4.438 ± 0.177 | 4.006 ± 0.093 | 4.200 ± 0.245 | 3.986 ± 0.189 | 4.064 ± 0.110 |
| Relative | 34.74 ± 1.75 | 36.67 ± 1.06 | 34.82 ± 1.05 | 35.08 ± 1.71 | 36.34 ± 1.04 | 40.51 ± 1.34* |
| Lung | | | | | | |
| Absolute | 0.876 ± 0.028 | 0.918 ± 0.038 | 0.898 ± 0.038 | 0.984 ± 0.048 | 0.886 ± 0.029 | 0.714 ± 0.012* |
| Relative | 7.59 ± 0.29 | 7.59 ± 0.28 | 7.83 ± 0.51 | 8.23 ± 0.37 | 8.09 ± 0.19 | 7.11 ± 0.11 |
| Thymus | | | | | | |
| Absolute | 0.275 ± 0.018 | 0.308 ± 0.019 | 0.271 ± 0.012 | 0.274 ± 0.026 | 0.249 ± 0.011 | 0.233 ± 0.027 |
| Relative | 2.38 ± 0.14 | 2.54 ± 0.11 | 2.36 ± 0.11 | 2.29 ± 0.20 | 2.27 ± 0.05 | 2.32 ± 0.26 |

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

** $P \leq 0.01$

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

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|------------------|----------------|------------------|------------------|------------------|------------------|------------------|
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Male | | | | | | |
| Necropsy body wt | 329 ± 5 | 343 ± 5 | 345 ± 4 | 333 ± 4 | 331 ± 4 | 301 ± 5** |
| Brain | | | | | | |
| Absolute | 1.889 ± 0.011 | 1.913 ± 0.018 | 1.895 ± 0.018 | 1.884 ± 0.014 | 1.886 ± 0.016 | 1.866 ± 0.017 |
| Relative | 5.75 ± 0.07 | 5.59 ± 0.06 | 5.50 ± 0.06 | 5.66 ± 0.05 | 5.71 ± 0.07 | 6.21 ± 0.08** |
| Heart | | | | | | |
| Absolute | 0.875 ± 0.021 | 0.913 ± 0.010 | 0.913 ± 0.017 | 0.964 ± 0.027* | 0.981 ± 0.035** | 0.904 ± 0.019 |
| Relative | 2.66 ± 0.05 | 2.67 ± 0.02 | 2.65 ± 0.04 | 2.90 ± 0.08** | 2.97 ± 0.10** | 3.00 ± 0.03** |
| R. Kidney | | | | | | |
| Absolute | 2.218 ± 0.055 | 2.369 ± 0.044 | 2.372 ± 0.051 | 2.512 ± 0.055** | 3.527 ± 0.087** | 2.915 ± 0.048** |
| Relative | 6.74 ± 0.14 | 6.91 ± 0.08 | 6.88 ± 0.11 | 7.54 ± 0.12** | 10.66 ± 0.20** | 9.68 ± 0.06** |
| Liver | | | | | | |
| Absolute | 11.587 ± 0.337 | 13.125 ± 0.407** | 13.494 ± 0.296** | 13.829 ± 0.354** | 14.825 ± 0.265** | 14.398 ± 0.402** |
| Relative | 35.19 ± 0.74 | 38.29 ± 0.98* | 39.16 ± 0.82** | 41.48 ± 0.72** | 44.92 ± 1.10** | 47.81 ± 1.01** |
| Lung | | | | | | |
| Absolute | 2.008 ± 0.064 | 2.033 ± 0.040 | 2.039 ± 0.041 | 2.175 ± 0.106 | 1.895 ± 0.072 | 1.890 ± 0.100 |
| Relative | 6.10 ± 0.17 | 5.94 ± 0.14 | 5.92 ± 0.12 | 6.56 ± 0.37 | 5.74 ± 0.24 | 6.28 ± 0.32 |
| R. Testis | | | | | | |
| Absolute | 1.384 ± 0.023 | 1.412 ± 0.029 | 1.343 ± 0.043 | 1.375 ± 0.031 | 1.472 ± 0.019 | 1.417 ± 0.020 |
| Relative | 4.21 ± 0.05 | 4.12 ± 0.08 | 3.90 ± 0.12 | 4.13 ± 0.10 | 4.45 ± 0.05* | 4.71 ± 0.05** |
| Thymus | | | | | | |
| Absolute | 0.236 ± 0.007 | 0.247 ± 0.014 | 0.256 ± 0.006 | 0.248 ± 0.007 | 0.244 ± 0.007 | 0.222 ± 0.006 |
| Relative | 0.72 ± 0.02 | 0.72 ± 0.04 | 0.74 ± 0.02 | 0.75 ± 0.02 | 0.74 ± 0.02 | 0.74 ± 0.02 |
| Female | | | | | | |
| Necropsy body wt | 189 ± 5 | 198 ± 3 | 197 ± 5 | 194 ± 4 | 177 ± 8 | 173 ± 4* |
| Brain | | | | | | |
| Absolute | 1.755 ± 0.017 | 1.795 ± 0.013 | 1.784 ± 0.021 | 1.763 ± 0.018 | 1.716 ± 0.035 | 1.704 ± 0.017 |
| Relative | 9.32 ± 0.16 | 9.07 ± 0.12 | 9.13 ± 0.25 | 9.08 ± 0.12 | 9.89 ± 0.57 | 9.87 ± 0.20 |
| Heart | | | | | | |
| Absolute | 0.596 ± 0.021 | 0.636 ± 0.012 | 0.644 ± 0.011 | 0.633 ± 0.014 | 0.597 ± 0.025 | 0.620 ± 0.010 |
| Relative | 3.15 ± 0.05 | 3.21 ± 0.04 | 3.28 ± 0.05 | 3.26 ± 0.05 | 3.38 ± 0.05** | 3.59 ± 0.06** |
| R. Kidney | | | | | | |
| Absolute | 1.326 ± 0.039 | 1.432 ± 0.028 | 1.485 ± 0.027* | 1.583 ± 0.038** | 1.710 ± 0.083** | 1.877 ± 0.043** |
| Relative | 7.01 ± 0.08 | 7.23 ± 0.13 | 7.57 ± 0.12** | 8.14 ± 0.12** | 9.66 ± 0.10** | 10.83 ± 0.13** |
| Liver | | | | | | |
| Absolute | 6.618 ± 0.260 | 6.687 ± 0.122 | 6.812 ± 0.114 | 7.044 ± 0.294 | 6.816 ± 0.331 | 7.567 ± 0.249* |
| Relative | 34.95 ± 0.84 | 33.75 ± 0.43 | 34.79 ± 0.80 | 36.17 ± 1.17 | 38.55 ± 0.78** | 43.58 ± 0.63** |
| Lung | | | | | | |
| Absolute | 1.411 ± 0.061 | 1.472 ± 0.023 | 1.501 ± 0.046 | 1.399 ± 0.037 | 1.307 ± 0.034 | 1.231 ± 0.030** |
| Relative | 7.47 ± 0.27 | 7.44 ± 0.12 | 7.64 ± 0.14 | 7.20 ± 0.15 | 7.49 ± 0.31 | 7.11 ± 0.11 |
| Thymus | | | | | | |
| Absolute | 0.204 ± 0.007 | 0.227 ± 0.004 | 0.227 ± 0.005 | 0.217 ± 0.007 | 0.188 ± 0.017 | 0.181 ± 0.006 |
| Relative | 1.08 ± 0.03 | 1.15 ± 0.02 | 1.16 ± 0.02 | 1.11 ± 0.03 | 1.05 ± 0.07 | 1.05 ± 0.04 |

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

** $P \leq 0.01$

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

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluation
in the 2-Year Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|------------------|----------------|----------------|-----------------|------------------|
| n | 10 | 10 | 10 | 10 |
| Male | | | | |
| Necropsy body wt | 490 ± 9 | 490 ± 10 | 485 ± 11 | 484 ± 11 |
| R. Kidney | | | | |
| Absolute | 1.487 ± 0.025 | 1.527 ± 0.045 | 1.592 ± 0.042 | 2.076 ± 0.077** |
| Relative | 3.04 ± 0.03 | 3.12 ± 0.07 | 3.28 ± 0.05* | 4.28 ± 0.11** |
| Liver | | | | |
| Absolute | 15.921 ± 0.258 | 16.155 ± 0.558 | 16.323 ± 0.507 | 16.912 ± 0.616 |
| Relative | 32.56 ± 0.40 | 32.92 ± 0.70 | 33.61 ± 0.50 | 34.96 ± 1.13 |
| Lung | | | | |
| Absolute | 2.283 ± 0.160 | 2.354 ± 0.074 | 2.178 ± 0.106 | 2.378 ± 0.154 |
| Relative | 4.63 ± 0.25 | 4.81 ± 0.14 | 4.49 ± 0.20 | 4.92 ± 0.31 |
| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
| Female | | | | |
| Necropsy body wt | 301 ± 8 | 299 ± 8 | 327 ± 4* | 304 ± 8 |
| R. Kidney | | | | |
| Absolute | 0.915 ± 0.020 | 0.933 ± 0.019 | 1.051 ± 0.026** | 1.178 ± 0.058** |
| Relative | 3.04 ± 0.05 | 3.13 ± 0.07 | 3.21 ± 0.07 | 3.88 ± 0.16** |
| Liver | | | | |
| Absolute | 8.759 ± 0.225 | 8.818 ± 0.239 | 9.922 ± 0.224** | 9.785 ± 0.226** |
| Relative | 29.08 ± 0.24 | 29.53 ± 0.43 | 30.32 ± 0.52 | 32.26 ± 0.52** |
| Lung | | | | |
| Absolute | 1.578 ± 0.058 | 1.468 ± 0.060 | 1.560 ± 0.064 | 1.487 ± 0.052 |
| Relative | 5.25 ± 0.17 | 4.91 ± 0.15 | 4.76 ± 0.17 | 4.90 ± 0.15 |

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

** $P \leq 0.01$

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

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Inhalation Study
of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|-------------------|---------------|---------------|---------------|---------------|----------------|-----------------|
| n | 5 | 5 | 5 | 5 | 5 | 5 |
| Male | | | | | | |
| Necropsy body wt | 21.2 ± 0.4 | 21.2 ± 0.6 | 20.5 ± 0.5 | 20.4 ± 1.1 | 22.0 ± 0.7 | 21.2 ± 0.9 |
| Brain | | | | | | |
| Absolute | 0.440 ± 0.005 | 0.456 ± 0.007 | 0.466 ± 0.009 | 0.452 ± 0.005 | 0.454 ± 0.009 | 0.494 ± 0.042 |
| Relative | 20.78 ± 0.37 | 21.59 ± 0.79 | 22.73 ± 0.39 | 22.28 ± 0.82 | 20.64 ± 0.41 | 23.23 ± 1.55 |
| Heart | | | | | | |
| Absolute | 0.140 ± 0.015 | 0.128 ± 0.014 | 0.138 ± 0.007 | 0.124 ± 0.011 | 0.128 ± 0.009 | 0.146 ± 0.008 |
| Relative | 6.60 ± 0.67 | 6.07 ± 0.72 | 6.74 ± 0.38 | 6.11 ± 0.61 | 5.82 ± 0.39 | 6.90 ± 0.38 |
| L. and R. Kidneys | | | | | | |
| Absolute | 0.408 ± 0.007 | 0.420 ± 0.013 | 0.404 ± 0.009 | 0.400 ± 0.017 | 0.428 ± 0.026 | 0.446 ± 0.019 |
| Relative | 19.26 ± 0.34 | 19.83 ± 0.56 | 19.71 ± 0.50 | 19.63 ± 0.52 | 19.43 ± 1.06 | 21.02 ± 0.53 |
| Liver | | | | | | |
| Absolute | 0.988 ± 0.031 | 1.002 ± 0.032 | 0.970 ± 0.044 | 0.986 ± 0.031 | 1.078 ± 0.052 | 1.032 ± 0.054 |
| Relative | 46.66 ± 1.65 | 47.34 ± 1.60 | 47.19 ± 1.25 | 48.48 ± 1.46 | 49.04 ± 2.51 | 48.53 ± 0.65 |
| Lung | | | | | | |
| Absolute | 0.190 ± 0.006 | 0.192 ± 0.008 | 0.208 ± 0.004 | 0.184 ± 0.005 | 0.204 ± 0.007 | 0.192 ± 0.008 |
| Relative | 8.97 ± 0.26 | 9.10 ± 0.53 | 10.16 ± 0.31 | 9.07 ± 0.39 | 9.26 ± 0.21 | 9.07 ± 0.34 |
| R. Testis | | | | | | |
| Absolute | 0.197 ± 0.012 | 0.202 ± 0.006 | 0.204 ± 0.009 | 0.202 ± 0.013 | 0.202 ± 0.007 | 0.199 ± 0.007 |
| Relative | 9.31 ± 0.54 | 9.52 ± 0.13 | 9.94 ± 0.37 | 9.85 ± 0.27 | 9.18 ± 0.17 | 9.38 ± 0.18 |
| Thymus | | | | | | |
| Absolute | 0.044 ± 0.009 | 0.042 ± 0.006 | 0.044 ± 0.007 | 0.047 ± 0.009 | 0.045 ± 0.006 | 0.045 ± 0.003 |
| Relative | 2.07 ± 0.41 | 1.99 ± 0.26 | 2.12 ± 0.33 | 2.27 ± 0.36 | 2.06 ± 0.26 | 2.13 ± 0.13 |
| Female | | | | | | |
| Necropsy body wt | 16.2 ± 0.4 | 16.7 ± 0.8 | 16.1 ± 0.2 | 16.8 ± 0.8 | 17.5 ± 0.6 | 17.5 ± 0.5 |
| Brain | | | | | | |
| Absolute | 0.458 ± 0.007 | 0.458 ± 0.020 | 0.458 ± 0.007 | 0.450 ± 0.018 | 0.464 ± 0.010 | 0.456 ± 0.002 |
| Relative | 28.33 ± 0.84 | 27.46 ± 0.86 | 28.49 ± 0.47 | 27.06 ± 1.79 | 26.55 ± 0.72 | 26.18 ± 0.79 |
| Heart | | | | | | |
| Absolute | 0.104 ± 0.004 | 0.120 ± 0.012 | 0.122 ± 0.006 | 0.104 ± 0.002 | 0.122 ± 0.007 | 0.128 ± 0.006 |
| Relative | 6.44 ± 0.31 | 7.13 ± 0.54 | 7.58 ± 0.34 | 6.24 ± 0.28 | 6.96 ± 0.33 | 7.33 ± 0.31 |
| L. and R. Kidneys | | | | | | |
| Absolute | 0.270 ± 0.009 | 0.284 ± 0.015 | 0.276 ± 0.010 | 0.278 ± 0.011 | 0.306 ± 0.015 | 0.316 ± 0.009* |
| Relative | 16.65 ± 0.24 | 16.97 ± 0.15 | 17.15 ± 0.53 | 16.59 ± 0.27 | 17.48 ± 0.72 | 18.10 ± 0.38 |
| Liver | | | | | | |
| Absolute | 0.722 ± 0.025 | 0.786 ± 0.031 | 0.736 ± 0.030 | 0.800 ± 0.058 | 0.858 ± 0.045* | 0.900 ± 0.047** |
| Relative | 44.66 ± 1.87 | 47.12 ± 1.04 | 45.72 ± 1.47 | 47.53 ± 1.89 | 48.88 ± 1.28 | 51.36 ± 1.35** |
| Lung | | | | | | |
| Absolute | 0.176 ± 0.009 | 0.178 ± 0.015 | 0.190 ± 0.004 | 0.182 ± 0.012 | 0.198 ± 0.011 | 0.184 ± 0.010 |
| Relative | 10.90 ± 0.67 | 10.59 ± 0.59 | 11.82 ± 0.32 | 10.83 ± 0.48 | 11.34 ± 0.70 | 10.52 ± 0.45 |
| Thymus | | | | | | |
| Absolute | 0.046 ± 0.003 | 0.054 ± 0.003 | 0.046 ± 0.002 | 0.056 ± 0.006 | 0.055 ± 0.004 | 0.063 ± 0.005* |
| Relative | 2.83 ± 0.19 | 3.23 ± 0.15 | 2.86 ± 0.15 | 3.31 ± 0.21 | 3.15 ± 0.23 | 3.63 ± 0.25* |

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

** $P \leq 0.01$

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

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|------------------|---------------|---------------|---------------|----------------------------|----------------------------|-----------------|
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Male | | | | | | |
| Necropsy body wt | 36.2 ± 0.7 | 35.3 ± 0.5 | 35.9 ± 0.8 | 34.6 ± 0.5* | 34.0 ± 0.3** | 32.1 ± 0.5** |
| Brain | | | | | | |
| Absolute | 0.464 ± 0.004 | 0.464 ± 0.006 | 0.465 ± 0.005 | 0.469 ± 0.002 | 0.464 ± 0.004 | 0.469 ± 0.006 |
| Relative | 12.84 ± 0.25 | 13.18 ± 0.21 | 12.99 ± 0.27 | 13.58 ± 0.22* | 13.64 ± 0.13* | 14.64 ± 0.24** |
| Heart | | | | | | |
| Absolute | 0.159 ± 0.004 | 0.157 ± 0.004 | 0.156 ± 0.004 | 0.158 ± 0.006 | 0.151 ± 0.005 | 0.145 ± 0.004 |
| Relative | 4.39 ± 0.12 | 4.45 ± 0.10 | 4.35 ± 0.10 | 4.57 ± 0.15 | 4.43 ± 0.13 | 4.52 ± 0.09 |
| R. Kidney | | | | | | |
| Absolute | 0.588 ± 0.009 | 0.606 ± 0.019 | 0.571 ± 0.017 | 0.573 ± 0.013 | 0.557 ± 0.012 | 0.550 ± 0.011 |
| Relative | 16.26 ± 0.30 | 17.18 ± 0.44 | 15.89 ± 0.31 | 16.56 ± 0.28 | 16.35 ± 0.27 | 17.15 ± 0.24 |
| Liver | | | | | | |
| Absolute | 1.672 ± 0.040 | 1.705 ± 0.047 | 1.687 ± 0.051 | 1.721 ± 0.045 | 1.622 ± 0.056 | 1.600 ± 0.049 |
| Relative | 46.13 ± 0.61 | 48.31 ± 0.91 | 46.93 ± 0.87 | 49.70 ± 0.86 | 47.58 ± 1.32 | 49.86 ± 1.28* |
| Lung | | | | | | |
| Absolute | 0.247 ± 0.004 | 0.242 ± 0.004 | 0.239 ± 0.007 | 0.232 ± 0.004* | 0.230 ± 0.003** | 0.216 ± 0.003** |
| Relative | 6.82 ± 0.08 | 6.87 ± 0.07 | 6.65 ± 0.13 | 6.71 ± 0.13 | 6.76 ± 0.09 | 6.75 ± 0.17 |
| R. Testis | | | | | | |
| Absolute | 0.117 ± 0.003 | 0.117 ± 0.002 | 0.116 ± 0.001 | 0.118 ± 0.001 | 0.114 ± 0.002 | 0.112 ± 0.003 |
| Relative | 3.24 ± 0.07 | 3.31 ± 0.04 | 3.25 ± 0.06 | 3.41 ± 0.07 | 3.36 ± 0.06 | 3.50 ± 0.10* |
| Thymus | | | | | | |
| Absolute | 0.032 ± 0.001 | 0.033 ± 0.002 | 0.033 ± 0.002 | 0.031 ± 0.001 | 0.031 ± 0.002 | 0.029 ± 0.001 |
| Relative | 0.89 ± 0.03 | 0.94 ± 0.04 | 0.93 ± 0.06 | 0.90 ± 0.04 | 0.90 ± 0.05 | 0.90 ± 0.05 |
| Female | | | | | | |
| Necropsy body wt | 31.5 ± 0.7 | 30.8 ± 0.8 | 30.9 ± 0.8 | 29.8 ± 0.6 | 30.7 ± 0.8 | 29.2 ± 0.5 |
| Brain | | | | | | |
| Absolute | 0.473 ± 0.004 | 0.468 ± 0.003 | 0.471 ± 0.006 | 0.479 ± 0.004 | 0.469 ± 0.005 | 0.474 ± 0.003 |
| Relative | 15.07 ± 0.32 | 15.28 ± 0.41 | 15.34 ± 0.39 | 16.11 ± 0.25 | 15.36 ± 0.35 | 16.26 ± 0.28 |
| Heart | | | | | | |
| Absolute | 0.131 ± 0.003 | 0.127 ± 0.002 | 0.126 ± 0.002 | 0.127 ± 0.002 | 0.127 ± 0.004 | 0.126 ± 0.002 |
| Relative | 4.17 ± 0.09 | 4.14 ± 0.09 | 4.10 ± 0.11 | 4.27 ± 0.06 | 4.15 ± 0.11 | 4.32 ± 0.11 |
| R. Kidney | | | | | | |
| Absolute | 0.384 ± 0.005 | 0.382 ± 0.014 | 0.372 ± 0.006 | 0.382 ± 0.009 | 0.382 ± 0.011 | 0.379 ± 0.010 |
| Relative | 12.22 ± 0.24 | 12.41 ± 0.38 | 12.11 ± 0.28 | 12.82 ± 0.19 | 12.46 ± 0.27 | 12.98 ± 0.33 |
| Liver | | | | | | |
| Absolute | 1.475 ± 0.043 | 1.460 ± 0.069 | 1.508 ± 0.026 | 1.506 ± 0.052 | 1.503 ± 0.063 | 1.498 ± 0.036 |
| Relative | 46.81 ± 0.97 | 47.30 ± 1.63 | 49.09 ± 1.28 | 50.44 ± 1.08 | 48.92 ± 1.44 | 51.32 ± 1.25* |
| Lung | | | | | | |
| Absolute | 0.238 ± 0.010 | 0.229 ± 0.006 | 0.249 ± 0.005 | 0.226 ± 0.004 | 0.236 ± 0.009 | 0.237 ± 0.011 |
| Relative | 7.53 ± 0.20 | 7.45 ± 0.17 | 8.10 ± 0.21 | 7.59 ± 0.13 | 7.69 ± 0.18 | 8.15 ± 0.44 |
| Thymus | | | | | | |
| Absolute | 0.041 ± 0.001 | 0.039 ± 0.002 | 0.039 ± 0.002 | 0.039 ± 0.002 ^b | 0.040 ± 0.002 ^c | 0.035 ± 0.003 |
| Relative | 1.29 ± 0.03 | 1.26 ± 0.05 | 1.26 ± 0.05 | 1.30 ± 0.06 ^b | 1.30 ± 0.06 ^c | 1.18 ± 0.10 |

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

** $P \leq 0.01$

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

^b n=9 ^c n=8

TABLE F6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 15-Month Interim Evaluation
in the 2-Year Inhalation Study of Tetrafluoroethylene ^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|------------------|---------------|---------------|---------------|---------------|
| n | 10 | 10 | 10 | 10 |
| Male | | | | |
| Necropsy body wt | 50.2 ± 1.6 | 50.8 ± 0.9 | 50.5 ± 1.6 | 48.5 ± 1.1 |
| R. Kidney | | | | |
| Absolute | 0.413 ± 0.010 | 0.406 ± 0.012 | 0.420 ± 0.013 | 0.413 ± 0.011 |
| Relative | 8.26 ± 0.13 | 8.00 ± 0.22 | 8.41 ± 0.40 | 8.52 ± 0.15 |
| Liver | | | | |
| Absolute | 2.845 ± 0.356 | 2.683 ± 0.231 | 2.455 ± 0.147 | 2.493 ± 0.175 |
| Relative | 59.58 ± 10.80 | 53.33 ± 5.04 | 49.30 ± 3.64 | 51.70 ± 3.96 |
| Lung | | | | |
| Absolute | 0.297 ± 0.013 | 0.316 ± 0.019 | 0.291 ± 0.013 | 0.308 ± 0.010 |
| Relative | 5.95 ± 0.24 | 6.22 ± 0.36 | 5.77 ± 0.15 | 6.36 ± 0.17 |
| Female | | | | |
| Necropsy body wt | 48.5 ± 1.5 | 50.8 ± 1.5 | 53.3 ± 3.0 | 48.3 ± 2.5 |
| R. Kidney | | | | |
| Absolute | 0.286 ± 0.011 | 0.270 ± 0.006 | 0.287 ± 0.009 | 0.278 ± 0.010 |
| Relative | 5.96 ± 0.31 | 5.33 ± 0.13 | 5.49 ± 0.26 | 5.83 ± 0.21 |
| Liver | | | | |
| Absolute | 1.886 ± 0.094 | 2.135 ± 0.173 | 2.210 ± 0.174 | 2.423 ± 0.321 |
| Relative | 39.02 ± 1.94 | 42.75 ± 4.42 | 41.34 ± 1.80 | 50.96 ± 7.10 |
| Lung | | | | |
| Absolute | 0.258 ± 0.006 | 0.283 ± 0.016 | 0.275 ± 0.008 | 0.278 ± 0.012 |
| Relative | 5.38 ± 0.24 | 5.59 ± 0.30 | 5.31 ± 0.34 | 5.90 ± 0.42 |

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). Differences from the control group were not significant by Williams' or Dunnett's test.

APPENDIX G
HEMATOLOGY, CLINICAL CHEMISTRY,
AND URINALYSIS RESULTS

TABLE G1 Hematology Data for Rats in the 16-Day Inhalation Study of Tetrafluoroethylene 264

TABLE G2 Hematology and Urinalysis Data for Rats in the 13-Week Inhalation Study of Tetrafluoroethylene 265

TABLE G3 Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 15-Month Interim Evaluation in the 2-Year Inhalation Study of Tetrafluoroethylene 267

TABLE G4 Hematology Data for Mice in the 16-Day Inhalation Study of Tetrafluoroethylene 269

TABLE G5 Hematology and Urinalysis Data for Mice in the 13-Week Inhalation Study of Tetrafluoroethylene 270

TABLE G1
Hematology Data for Rats in the 16-Day Inhalation Study of Tetrafluoroethylene ^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|--------------|--------------|--------------|--------------|---------------|--------------|
| Male | | | | | | |
| n | 5 | 5 | 5 | 5 | 4 | 5 |
| Hematocrit (%) | 43.0 ± 0.3 | 42.4 ± 0.6 | 41.7 ± 0.2 | 42.1 ± 0.4 | 41.9 ± 0.3 | 42.6 ± 0.5 |
| Hemoglobin (g/dL) | 16.2 ± 0.2 | 16.0 ± 0.3 | 15.7 ± 0.1 | 15.9 ± 0.2 | 15.8 ± 0.2 | 16.0 ± 0.2 |
| Erythrocytes (10 ⁶ /μL) | 7.93 ± 0.05 | 7.70 ± 0.14 | 7.67 ± 0.12 | 7.68 ± 0.10 | 7.72 ± 0.05 | 7.77 ± 0.10 |
| Reticulocytes (per 5 oil immersion fields) | 53.00 ± 8.56 | 54.40 ± 4.07 | 66.80 ± 8.29 | 70.00 ± 5.52 | 68.75 ± 7.22 | 69.20 ± 5.44 |
| Nucleated erythrocytes (10 ³ /μL) | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Mean cell volume (fL) | 54.2 ± 0.2 | 55.0 ± 0.3 | 54.6 ± 0.7 | 55.0 ± 0.6 | 54.3 ± 0.3 | 55.0 ± 0.6 |
| Mean cell hemoglobin (pg) | 20.5 ± 0.1 | 20.8 ± 0.2 | 20.5 ± 0.3 | 20.8 ± 0.2 | 20.4 ± 0.2 | 20.6 ± 0.2 |
| Mean cell hemoglobin concentration (g/dL) | 37.8 ± 0.1 | 37.7 ± 0.2 | 37.7 ± 0.1 | 37.8 ± 0.1 | 37.6 ± 0.1 | 37.5 ± 0.1 |
| Platelets (10 ³ /μL) | 641.4 ± 10.3 | 699.4 ± 22.4 | 603.0 ± 74.8 | 677.6 ± 15.2 | 745.3 ± 20.3* | 641.8 ± 31.4 |
| Leukocytes (10 ³ /μL) | 3.66 ± 0.17 | 4.12 ± 0.09 | 4.26 ± 0.49 | 3.68 ± 0.35 | 4.20 ± 0.67 | 4.18 ± 0.32 |
| Segmented neutrophils (10 ³ /μL) | 0.70 ± 0.09 | 0.71 ± 0.09 | 0.82 ± 0.16 | 0.70 ± 0.14 | 0.60 ± 0.04 | 0.65 ± 0.06 |
| Lymphocytes (10 ³ /μL) | 2.92 ± 0.16 | 3.35 ± 0.03 | 3.38 ± 0.41 | 2.91 ± 0.20 | 3.54 ± 0.67 | 3.52 ± 0.34 |
| Monocytes (10 ³ /μL) | 0.02 ± 0.02 | 0.03 ± 0.02 | 0.06 ± 0.04 | 0.07 ± 0.03 | 0.03 ± 0.01 | 0.01 ± 0.01 |
| Eosinophils (10 ³ /μL) | 0.01 ± 0.01 | 0.04 ± 0.01 | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.04 ± 0.02 | 0.00 ± 0.00 |
| Female | | | | | | |
| n | 5 | 5 | 5 | 5 | 5 | 5 |
| Hematocrit (%) | 45.6 ± 0.3 | 45.7 ± 0.5 | 45.8 ± 0.1 | 46.9 ± 0.4 | 46.6 ± 0.3 | 46.2 ± 0.6 |
| Hemoglobin (g/dL) | 16.8 ± 0.1 | 16.8 ± 0.2 | 16.9 ± 0.1 | 17.3 ± 0.1 | 17.2 ± 0.2 | 17.1 ± 0.3 |
| Erythrocytes (10 ⁶ /μL) | 8.11 ± 0.18 | 8.16 ± 0.11 | 8.12 ± 0.12 | 8.28 ± 0.09 | 8.49 ± 0.14 | 8.34 ± 0.11 |
| Reticulocytes (per 5 oil immersion fields) | 46.00 ± 7.77 | 42.00 ± 5.65 | 32.20 ± 1.59 | 37.20 ± 3.09 | 35.00 ± 3.48 | 31.40 ± 1.33 |
| Nucleated erythrocytes (10 ³ /μL) | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Mean cell volume (fL) | 56.4 ± 1.3 | 56.0 ± 0.6 | 56.6 ± 1.0 | 56.8 ± 0.4 | 55.0 ± 0.6 | 55.6 ± 0.4 |
| Mean cell hemoglobin (pg) | 20.8 ± 0.5 | 20.6 ± 0.1 | 20.8 ± 0.4 | 20.9 ± 0.1 | 20.3 ± 0.2 | 20.5 ± 0.2 |
| Mean cell hemoglobin concentration (g/dL) | 36.9 ± 0.1 | 36.7 ± 0.2 | 36.9 ± 0.2 | 37.0 ± 0.1 | 36.9 ± 0.1 | 36.9 ± 0.2 |
| Platelets (10 ³ /μL) | 626.8 ± 22.5 | 705.6 ± 17.5 | 626.2 ± 12.7 | 650.2 ± 49.6 | 552.2 ± 20.8 | 599.8 ± 14.1 |
| Leukocytes (10 ³ /μL) | 4.80 ± 0.10 | 4.16 ± 0.46 | 3.96 ± 0.27 | 4.72 ± 0.48 | 4.82 ± 0.77 | 4.18 ± 0.44 |
| Segmented neutrophils (10 ³ /μL) | 0.81 ± 0.10 | 0.70 ± 0.14 | 0.55 ± 0.15 | 0.70 ± 0.03 | 1.07 ± 0.22 | 1.08 ± 0.23 |
| Lymphocytes (10 ³ /μL) | 3.86 ± 0.16 | 3.37 ± 0.42 | 3.29 ± 0.30 | 3.86 ± 0.41 | 3.68 ± 0.60 | 2.88 ± 0.31 |
| Monocytes (10 ³ /μL) | 0.11 ± 0.02 | 0.08 ± 0.03 | 0.10 ± 0.04 | 0.11 ± 0.04 | 0.07 ± 0.02 | 0.18 ± 0.04 |
| Eosinophils (10 ³ /μL) | 0.02 ± 0.01 | 0.02 ± 0.02 | 0.02 ± 0.01 | 0.05 ± 0.02 | 0.01 ± 0.01 | 0.04 ± 0.01 |

* Significantly different (P≤0.05) from the control group by Dunn's test

^a Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE G2
Hematology and Urinalysis Data for Rats in the 13-Week Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|----------------------------|------------------------------|----------------------------|----------------|------------------|--------------------------|
| Male | | | | | | |
| Hematology | | | | | | |
| n | 10 | 9 | 9 | 10 | 9 | 10 |
| Hematocrit (%) | 47.3 ± 0.3 | 45.5 ± 0.3** | 45.9 ± 0.4** | 44.9 ± 0.6** | 44.3 ± 0.3** | 44.3 ± 0.3** |
| Hemoglobin (g/dL) | 15.8 ± 0.1 | 15.2 ± 0.1** | 15.3 ± 0.1** | 14.8 ± 0.2** | 14.6 ± 0.1** | 14.5 ± 0.1** |
| Erythrocytes (10 ⁶ /μL) | 9.45 ± 0.06 | 9.11 ± 0.07** | 9.22 ± 0.09* | 9.02 ± 0.12** | 8.91 ± 0.08** | 8.80 ± 0.07** |
| Reticulocytes (10 ³ /μL) | 91.2 ± 47.5 | 118.8 ± 60.3 | 109.9 ± 41.7 | 84.2 ± 44.1 | 116.9 ± 82.7 | 87.0 ± 61.2 |
| Mean cell volume (fL) | 50.2 ± 0.3 | 49.9 ± 0.2 | 49.7 ± 0.2 | 49.9 ± 0.2 | 49.8 ± 0.2 | 50.3 ± 0.2 |
| Mean cell hemoglobin (pg) | 16.7 ± 0.1 | 16.7 ± 0.1 | 16.6 ± 0.1 | 16.5 ± 0.2* | 16.4 ± 0.1** | 16.5 ± 0.1* |
| Mean cell hemoglobin concentration (g/dL) | 33.4 ± 0.1 | 33.3 ± 0.1 | 33.3 ± 0.1 | 33.0 ± 0.3* | 33.0 ± 0.1* | 32.8 ± 0.1** |
| Platelets (10 ³ /μL) | 515.4 ± 8.6 | 530.1 ± 15.8 | 540.6 ± 23.8* | 599.4 ± 21.8** | 581.1 ± 10.6** | 637.4 ± 12.4** |
| Leukocytes (10 ³ /μL) | 6.38 ± 0.20 | 6.27 ± 0.20 | 6.43 ± 0.27 | 6.59 ± 0.31 | 6.02 ± 0.45 | 6.59 ± 0.27 |
| Segmented neutrophils (10 ³ /μL) | 1.06 ± 0.10 | 1.16 ± 0.11 | 0.89 ± 0.07 | 0.91 ± 0.10 | 0.86 ± 0.11 | 0.97 ± 0.08 |
| Lymphocytes (10 ³ /μL) | 5.07 ± 0.18 | 4.82 ± 0.26 | 5.33 ± 0.19 | 5.51 ± 0.29 | 4.95 ± 0.38 | 5.39 ± 0.21 |
| Monocytes (10 ³ /μL) | 0.22 ± 0.05 | 0.18 ± 0.04 | 0.16 ± 0.06 | 0.09 ± 0.03 | 0.17 ± 0.04 | 0.19 ± 0.05 |
| Eosinophils (10 ³ /μL) | 0.04 ± 0.02 | 0.10 ± 0.01* | 0.05 ± 0.02 | 0.07 ± 0.02 | 0.04 ± 0.02 | 0.04 ± 0.01 |
| Urinalysis | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Fluoride (μg/16 hr) | 7.98 ± 0.37 | 35.68 ± 1.82** | 45.87 ± 2.42** | 69.91 ± 5.63** | 122.41 ± 12.02** | 205.45 ± 16.71** |
| Glucose (mg/16 hr) | 0.09 ± 0.01 ^b | 0.02 ± 0.00** ^b | 0.08 ± 0.02 | 0.04 ± 0.01 | 0.05 ± 0.02 | 2.81 ± 1.27 ^b |
| Protein (mg/16 hr) | 14 ± 1 | 17 ± 1** | 19 ± 1** | 23 ± 2** | 23 ± 1** | 35 ± 2** |
| Volume (mL/16 hr) | 24.8 ± 2.4 | 21.5 ± 3.1 | 27.0 ± 2.1 | 24.0 ± 2.8 | 28.7 ± 2.0 | 38.6 ± 4.5 |
| Volume (mL) (concentrated) | 0.9 ± 0.2 ^b | 0.8 ± 0.1 ^b | 0.9 ± 0.2 ^b | 1.1 ± 0.2 | 1.2 ± 0.1 | 1.1 ± 0.2 |
| Specific gravity | 1.010 ± 0.001 | 1.014 ± 0.002 | 1.009 ± 0.001 | 1.012 ± 0.002 | 1.009 ± 0.001 | 1.009 ± 0.001 |
| Specific gravity (concentrated) | 1.037 ± 0.006 ^b | 1.064 ± 0.001** ^b | 1.060 ± 0.002 ^b | 1.058 ± 0.003* | 1.055 ± 0.002 | 1.060 ± 0.002* |

TABLE G2
Hematology and Urinalysis Data for Rats in the 13-Week Inhalation Study of Tetrafluoroethylene (continued)

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|---|----------------------------|----------------------------|----------------------------|-----------------------------|----------------------------|------------------------------|
| Female | | | | | | |
| Hematology | | | | | | |
| n | 10 | 10 | 10 | 10 | 9 | 10 |
| Hematocrit (%) | 47.5 ± 0.4 | 47.3 ± 0.3 | 46.8 ± 0.5 | 47.2 ± 0.3 | 48.0 ± 0.7 | 45.3 ± 0.3** |
| Hemoglobin (g/dL) | 15.7 ± 0.2 | 15.7 ± 0.2 | 15.6 ± 0.2 | 15.6 ± 0.1 | 15.9 ± 0.2 | 14.9 ± 0.1** |
| Erythrocytes (10 ⁶ /μL) | 8.67 ± 0.08 | 8.67 ± 0.08 | 8.67 ± 0.09 | 8.66 ± 0.05 | 8.79 ± 0.08 | 8.34 ± 0.09* |
| Reticulocytes (10 ³ /μL) | 82.8 ± 36.4 | 88.6 ± 22.1 | 71.5 ± 30.5 | 86.6 ± 33.0 | 67.2 ± 41.2 | 96.4 ± 33.3 |
| Mean cell volume (fL) | 54.9 ± 0.2 | 54.4 ± 0.2 | 54.1 ± 0.2* | 54.5 ± 0.2 | 54.7 ± 0.3 | 54.5 ± 0.4 |
| Mean cell hemoglobin (pg) | 18.1 ± 0.1 | 18.1 ± 0.1 | 17.9 ± 0.1 | 18.0 ± 0.1 | 18.0 ± 0.1 | 17.9 ± 0.2** |
| Mean cell hemoglobin concentration (g/dL) | 33.2 ± 0.1 | 33.1 ± 0.2 | 33.3 ± 0.2 | 33.1 ± 0.1 | 33.1 ± 0.1 | 32.8 ± 0.1 |
| Platelets (10 ³ /μL) | 589.0 ± 31.3 | 584.7 ± 11.8 | 571.1 ± 17.2 | 585.5 ± 14.3 | 614.4 ± 25.8 | 665.5 ± 19.1 |
| Leukocytes (10 ³ /μL) | 6.19 ± 0.43 | 5.07 ± 0.15 | 5.55 ± 0.28 | 5.35 ± 0.23 | 5.74 ± 0.33 | 4.84 ± 0.26* |
| Segmented neutrophils (10 ³ /μL) | 0.66 ± 0.05 | 0.65 ± 0.09 | 0.86 ± 0.07 | 0.67 ± 0.09 | 0.82 ± 0.12 | 0.76 ± 0.06 |
| Lymphocytes (10 ³ /μL) | 5.39 ± 0.43 | 4.26 ± 0.20 | 4.58 ± 0.29 | 4.51 ± 0.22 | 4.81 ± 0.33 | 3.95 ± 0.22* |
| Monocytes (10 ³ /μL) | 0.11 ± 0.03 | 0.10 ± 0.03 | 0.08 ± 0.02 | 0.13 ± 0.04 | 0.10 ± 0.03 | 0.11 ± 0.03 |
| Eosinophils (10 ³ /μL) | 0.03 ± 0.02 | 0.05 ± 0.01 | 0.03 ± 0.02 | 0.04 ± 0.02 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| Urinalysis | | | | | | |
| n | 10 | 10 | 10 | 10 | 8 | 10 |
| Fluoride (μg/16 hr) | 4.33 ± 0.17 | 20.71 ± 1.12** | 32.30 ± 1.22** | 49.86 ± 4.38** ^b | 73.80 ± 4.68** | 118.76 ± 5.00** ^c |
| Glucose (mg/16 hr) | 0.12 ± 0.06 | 0.10 ± 0.03 | 0.18 ± 0.07 | 0.09 ± 0.02 | 0.21 ± 0.04** | 0.06 ± 0.01 |
| Protein (mg/16 hr) | 1 ± 0 | 1 ± 0 | 1 ± 0 | 1 ± 0 | 2 ± 0** | 2 ± 0** |
| Volume (mL/16 hr) | 11.9 ± 1.5 | 18.1 ± 2.1* | 20.0 ± 3.1* | 23.3 ± 1.9** | 34.3 ± 3.7** | 38.5 ± 3.1** |
| Volume (mL) (concentrated) | 0.5 ± 0.2 ^b | 0.8 ± 0.2 ^c | 0.6 ± 0.2 ^b | 0.6 ± 0.2 ^d | 0.4 ± 0.2 ^c | 0.4 ± 0.1 |
| Specific gravity | 1.015 ± 0.002 | 1.009 ± 0.001* | 1.010 ± 0.001 | 1.008 ± 0.001** | 1.005 ± 0.001** | 1.006 ± 0.000** |
| Specific gravity (concentrated) | 1.049 ± 0.007 ^b | 1.053 ± 0.013 ^c | 1.064 ± 0.005 ^b | 1.062 ± 0.012 ^d | 1.079 ± 0.011 ^c | 1.057 ± 0.007 |

* Significantly different (P<0.05) from the control group by Dunn's or Shirley's test

** P<0.01

^a Mean ± standard error. Statistical tests were performed on unrounded data.

^b n=9

^c n=8

^d n=7

^e n=6

TABLE G3
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 15-Month Interim Evaluation
in the 2-Year Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 156 ppm | 312 ppm | 625 ppm |
|--|----------------------------|----------------------------|----------------------------|----------------------------|
| Male | | | | |
| n | 10 | 10 | 10 | 10 |
| Hematology | | | | |
| Hematocrit (automated) (%) | 44.0 ± 0.9 | 45.0 ± 0.7 | 45.0 ± 1.0 | 42.1 ± 0.9 |
| Hematocrit (manual) (%) | 43.0 ± 0.8 | 43.5 ± 0.8 | 43.7 ± 0.9 | 41.1 ± 0.8 |
| Hemoglobin (g/dL) | 14.1 ± 0.3 | 14.2 ± 0.2 | 14.5 ± 0.3 | 13.4 ± 0.3 |
| Erythrocytes (10 ⁶ /μL) | 8.37 ± 0.13 | 8.40 ± 0.15 | 8.54 ± 0.20 | 7.79 ± 0.26 |
| Reticulocytes (10 ⁶ /μL) | 0.30 ± 0.03 | 0.30 ± 0.02 | 0.30 ± 0.02 | 0.36 ± 0.08 |
| Nucleated erythrocytes (10 ³ /μL) | 0.12 ± 0.03 | 0.13 ± 0.02 | 0.09 ± 0.02 | 0.27 ± 0.14 |
| Mean cell volume (fL) | 52.7 ± 0.8 | 53.6 ± 0.3 | 52.8 ± 0.4 | 54.2 ± 0.9 |
| Mean cell hemoglobin (pg) | 16.9 ± 0.3 | 16.9 ± 0.1 | 17.0 ± 0.1 | 17.3 ± 0.3 |
| Mean cell hemoglobin concentration (g/dL) | 32.2 ± 0.1 | 31.6 ± 0.1* | 32.2 ± 0.1 | 31.9 ± 0.1 |
| Platelets (10 ³ /μL) | 535.7 ± 21.9 | 531.8 ± 8.2 | 562.0 ± 16.4 | 520.8 ± 32.4 |
| Leukocytes (10 ³ /μL) | 3.45 ± 0.83 | 2.89 ± 0.56 | 2.19 ± 0.13 | 3.06 ± 0.41 |
| Segmented neutrophils (10 ³ /μL) | 1.76 ± 0.49 | 1.11 ± 0.21 | 0.96 ± 0.08 | 1.11 ± 0.12 |
| Lymphocytes (10 ³ /μL) | 1.66 ± 0.35 | 1.74 ± 0.36 | 1.21 ± 0.12 | 1.89 ± 0.36 |
| Monocytes (10 ³ /μL) | 0.01 ± 0.00 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.01 ± 0.01 |
| Eosinophils (10 ³ /μL) | 0.02 ± 0.01 | 0.03 ± 0.02 | 0.01 ± 0.01 | 0.05 ± 0.01 |
| Clinical Chemistry | | | | |
| Blood urea nitrogen (mg/dL) | 18.4 ± 0.3 | 19.7 ± 0.7 | 19.3 ± 0.5 | 20.6 ± 0.7* |
| Creatinine (mg/dL) | 0.63 ± 0.02 | 0.67 ± 0.03 | 0.59 ± 0.02 | 0.67 ± 0.02 |
| Sodium (mEq/L) | 152 ± 1 | 152 ± 1 | 151 ± 1 | 151 ± 1 |
| Potassium (mEq/L) | 5.2 ± 0.1 | 5.1 ± 0.1 | 5.1 ± 0.1 | 5.3 ± 0.1 |
| Chloride (mEq/L) | 107 ± 1 | 106 ± 1 | 107 ± 0 | 106 ± 1 |
| Calcium (mg/dL) | 11.42 ± 0.15 | 11.48 ± 0.15 | 11.78 ± 0.12 | 11.48 ± 0.15 |
| Phosphorus (mg/dL) | 5.9 ± 0.3 | 6.0 ± 0.3 | 6.4 ± 0.2 | 6.4 ± 0.2 |
| Urinalysis | | | | |
| Glucose (mg/16 hr) | 1.5 ± 0.1 | 1.4 ± 0.1 | 1.0 ± 0.1** | 1.0 ± 0.0** |
| Protein (mg/16 hr) | 19 ± 2 | 17 ± 2 | 14 ± 1 | 27 ± 4 |
| Volume (mL/16 hr) | | | | |
| Week 63 | 14.7 ± 2.8 | 9.0 ± 0.8 | 8.3 ± 0.6* | 12.9 ± 0.8 |
| Week 64 | 0.7 ± 0.2 | 0.8 ± 0.2 | 1.0 ± 0.3 | 1.4 ± 0.3 |
| Specific gravity | | | | |
| Week 63 | 1.022 ± 0.002 | 1.029 ± 0.001 | 1.026 ± 0.001 | 1.023 ± 0.001 |
| Week 64 | 1.048 ± 0.006 ^b | 1.045 ± 0.007 ^b | 1.039 ± 0.008 ^c | 1.045 ± 0.006 ^d |

TABLE G3
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 15-Month Interim Evaluation
in the 2-Year Inhalation Study of Tetrafluoroethylene (continued)

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm |
|--|----------------------------|----------------------------|----------------------------|----------------------------|
| Female | | | | |
| n | 10 | 10 | 10 | 10 |
| Hematology | | | | |
| Hematocrit (automated) (%) | 44.4 ± 0.4 | 44.9 ± 0.5 | 44.0 ± 0.4 | 43.9 ± 0.5 |
| Hematocrit (manual) (%) | 42.9 ± 0.4 | 43.4 ± 0.4 | 42.6 ± 0.4 | 42.9 ± 0.5 |
| Hemoglobin (g/dL) | 14.3 ± 0.2 | 14.4 ± 0.2 | 14.1 ± 0.1 | 14.0 ± 0.2 |
| Erythrocytes (10 ⁶ /μL) | 7.82 ± 0.07 | 7.95 ± 0.07 | 7.81 ± 0.09 | 7.87 ± 0.08 |
| Reticulocytes (10 ⁶ /μL) | 0.23 ± 0.02 | 0.25 ± 0.01 | 0.25 ± 0.01 | 0.27 ± 0.01* |
| Nucleated erythrocytes (10 ³ /μL) | 0.09 ± 0.02 | 0.07 ± 0.01 | 0.08 ± 0.01 | 0.06 ± 0.01 |
| Mean cell volume (fL) | 56.9 ± 0.2 | 56.4 ± 0.2 | 56.3 ± 0.3 | 55.8 ± 0.2** |
| Mean cell hemoglobin (pg) | 18.3 ± 0.1 | 18.1 ± 0.1 | 18.1 ± 0.1 | 17.8 ± 0.1** |
| Mean cell hemoglobin concentration (g/dL) | 32.1 ± 0.2 | 32.0 ± 0.1 | 32.0 ± 0.1 | 32.0 ± 0.1 |
| Platelets (10 ³ /μL) | 463.3 ± 16.9 | 492.4 ± 11.8 | 479.8 ± 12.6 | 491.0 ± 9.8 |
| Leukocytes (10 ³ /μL) | 1.45 ± 0.18 | 1.51 ± 0.15 | 1.86 ± 0.28 | 1.89 ± 0.16* |
| Segmented neutrophils (10 ³ /μL) | 0.43 ± 0.06 | 0.47 ± 0.05 | 0.65 ± 0.10* | 0.70 ± 0.09** |
| Lymphocytes (10 ³ /μL) | 1.00 ± 0.14 | 1.01 ± 0.11 | 1.19 ± 0.19 | 1.16 ± 0.12 |
| Monocytes (10 ³ /μL) | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| Eosinophils (10 ³ /μL) | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 |
| Clinical Chemistry | | | | |
| Blood urea nitrogen (mg/dL) | 17.6 ± 0.8 | 17.4 ± 0.6 | 17.6 ± 0.7 | 17.7 ± 0.5 |
| Creatinine (mg/dL) | 0.67 ± 0.03 | 0.64 ± 0.02 | 0.61 ± 0.02 | 0.62 ± 0.03 |
| Sodium (mEq/L) | 148 ± 0 | 148 ± 1 | 148 ± 1 | 147 ± 1 |
| Potassium (mEq/L) | 5.2 ± 0.1 | 5.1 ± 0.1 | 5.0 ± 0.1 | 5.2 ± 0.1 |
| Chloride (mEq/L) | 100 ± 1 | 102 ± 1 | 99 ± 0 | 100 ± 1 |
| Calcium (mg/dL) | 11.55 ± 0.09 | 11.44 ± 0.17 | 11.26 ± 0.12 | 11.18 ± 0.17 |
| Phosphorus (mg/dL) | 6.2 ± 0.3 | 5.9 ± 0.4 | 6.0 ± 0.3 | 6.5 ± 0.2 |
| Urinalysis | | | | |
| Glucose (mg/16 hr) | 0.8 ± 0.1 | 0.8 ± 0.0 | 0.7 ± 0.1 | 0.8 ± 0.4 |
| Protein (mg/16 hr) | 4 ± 1 | 4 ± 1 | 5 ± 1 | 5 ± 1 |
| Volume (mL/16 hr) | | | | |
| Week 63 | 9.6 ± 2.2 | 6.1 ± 0.5 | 7.4 ± 0.8 | 10.1 ± 0.7 |
| Week 64 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| Specific gravity | | | | |
| Week 63 | 1.019 ± 0.002 | 1.027 ± 0.002* | 1.021 ± 0.002 | 1.019 ± 0.001 |
| Week 64 | 1.038 ± 0.012 ^e | 1.046 ± 0.006 ^c | 1.060 ± 0.002 ^e | 1.061 ± 0.007 ^e |

* Significantly different (P<0.05) from the control group by Dunn's or Shirley's test

** P<0.01

^a Mean ± standard error. Statistical tests were performed on unrounded data.

^b n=6

^c n=5

^d n=9

^e n=3

TABLE G4
Hematology Data for Mice in the 16-Day Inhalation Study of Tetrafluoroethylene ^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|--------------|---------------|--------------|--------------|--------------|--------------|
| Male | | | | | | |
| n | 5 | 5 | 5 | 5 | 5 | 5 |
| Hematocrit (%) | 44.3 ± 0.4 | 45.2 ± 0.9 | 44.4 ± 0.4 | 43.6 ± 0.5 | 43.5 ± 0.5 | 43.8 ± 0.3 |
| Hemoglobin (g/dL) | 17.7 ± 0.2 | 18.0 ± 0.4 | 17.6 ± 0.1 | 17.0 ± 0.3 | 17.2 ± 0.3 | 17.3 ± 0.2 |
| Erythrocytes (10 ⁶ /μL) | 8.24 ± 0.09 | 8.46 ± 0.17 | 8.38 ± 0.06 | 8.14 ± 0.09 | 7.94 ± 0.07 | 8.18 ± 0.03 |
| Reticulocytes (per 5 oil immersion fields) | 29.80 ± 2.99 | 15.20 ± 4.53* | 21.80 ± 5.03 | 21.40 ± 5.77 | 23.20 ± 4.31 | 24.00 ± 1.55 |
| Nucleated erythrocytes (10 ³ /μL) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Mean cell volume (fL) | 53.8 ± 0.4 | 53.6 ± 0.6 | 53.0 ± 0.3 | 53.6 ± 0.2 | 55.0 ± 0.0 | 53.6 ± 0.2 |
| Mean cell hemoglobin (pg) | 21.4 ± 0.2 | 21.3 ± 0.2 | 21.0 ± 0.1 | 20.9 ± 0.2 | 21.7 ± 0.2 | 21.2 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | 39.8 ± 0.3 | 39.9 ± 0.2 | 39.6 ± 0.1 | 39.1 ± 0.5 | 39.6 ± 0.4 | 39.6 ± 0.3 |
| Platelets (10 ³ /μL) | 805.0 ± 39.9 | 782.2 ± 16.7 | 759.8 ± 4.1 | 778.0 ± 24.1 | 815.6 ± 70.7 | 799.4 ± 34.8 |
| Leukocytes (10 ³ /μL) | 1.62 ± 0.26 | 1.54 ± 0.41 | 1.92 ± 0.59 | 1.24 ± 0.38 | 1.10 ± 0.30 | 1.90 ± 0.23 |
| Segmented neutrophils (10 ³ /μL) | 0.48 ± 0.09 | 0.22 ± 0.04 | 0.72 ± 0.35 | 0.26 ± 0.08 | 0.17 ± 0.08 | 0.52 ± 0.08 |
| Lymphocytes (10 ³ /μL) | 1.10 ± 0.30 | 1.29 ± 0.35 | 1.16 ± 0.26 | 0.92 ± 0.33 | 0.90 ± 0.23 | 1.33 ± 0.20 |
| Monocytes (10 ³ /μL) | 0.04 ± 0.02 | 0.03 ± 0.02 | 0.05 ± 0.02 | 0.05 ± 0.03 | 0.03 ± 0.01 | 0.05 ± 0.03 |
| Eosinophils (10 ³ /μL) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Female | | | | | | |
| n | 4 | 5 | 5 | 5 | 5 | 5 |
| Hematocrit (%) | 44.6 ± 0.7 | 43.8 ± 0.8 | 43.5 ± 0.6 | 43.3 ± 0.6 | 44.3 ± 0.3 | 43.3 ± 0.4 |
| Hemoglobin (g/dL) | 17.5 ± 0.3 | 17.2 ± 0.3 | 17.0 ± 0.3 | 17.1 ± 0.2 | 17.4 ± 0.1 | 17.0 ± 0.1 |
| Erythrocytes (10 ⁶ /μL) | 8.24 ± 0.12 | 8.16 ± 0.15 | 7.95 ± 0.11 | 8.08 ± 0.14 | 8.22 ± 0.06 | 8.07 ± 0.07 |
| Reticulocytes (per 5 oil immersion fields) | 25.00 ± 1.91 | 29.00 ± 3.39 | 21.40 ± 3.16 | 15.00 ± 2.02 | 29.40 ± 3.72 | 20.20 ± 4.18 |
| Nucleated erythrocytes (10 ³ /μL) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Mean cell volume (fL) | 54.3 ± 0.3 | 53.6 ± 0.4 | 54.6 ± 0.2 | 53.6 ± 0.2 | 53.8 ± 0.2 | 53.8 ± 0.2 |
| Mean cell hemoglobin (pg) | 21.2 ± 0.1 | 21.1 ± 0.2 | 21.4 ± 0.2 | 21.2 ± 0.3 | 21.1 ± 0.1 | 21.1 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | 39.1 ± 0.1 | 39.3 ± 0.1 | 39.2 ± 0.2 | 39.6 ± 0.4 | 39.2 ± 0.3 | 39.3 ± 0.1 |
| Platelets (10 ³ /μL) | 797.3 ± 26.7 | 764.4 ± 32.2 | 734.2 ± 25.6 | 775.0 ± 27.0 | 785.6 ± 30.2 | 797.6 ± 24.9 |
| Leukocytes (10 ³ /μL) | 0.85 ± 0.22 | 0.76 ± 0.08 | 0.64 ± 0.13 | 0.94 ± 0.18 | 0.98 ± 0.22 | 0.86 ± 0.16 |
| Segmented neutrophils (10 ³ /μL) | 0.30 ± 0.04 | 0.14 ± 0.03 | 0.24 ± 0.04 | 0.29 ± 0.13 | 0.38 ± 0.18 | 0.13 ± 0.02 |
| Lymphocytes (10 ³ /μL) | 0.55 ± 0.21 | 0.62 ± 0.07 | 0.39 ± 0.12 | 0.64 ± 0.12 | 0.59 ± 0.07 | 0.72 ± 0.15 |
| Monocytes (10 ³ /μL) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| Eosinophils (10 ³ /μL) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

* Significantly different (P≤0.05) from the control group by Dunn's test

^a Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE G5
Hematology and Urinalysis Data for Mice in the 13-Week Inhalation Study of Tetrafluoroethylene ^a

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|--------------------------|-----------------------------|-----------------------------|----------------|-----------------|-----------------|
| Male | | | | | | |
| Hematology | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematocrit (%) | 50.6 ± 0.4 | 50.0 ± 0.5 | 50.2 ± 0.4 | 50.4 ± 0.7 | 47.9 ± 0.4** | 46.3 ± 0.5** |
| Hemoglobin (g/dL) | 17.0 ± 0.2 | 16.7 ± 0.1 | 16.7 ± 0.1 | 16.7 ± 0.1 | 16.0 ± 0.1** | 15.4 ± 0.2** |
| Erythrocytes (10 ⁶ /μL) | 10.51 ± 0.10 | 10.35 ± 0.09 | 10.42 ± 0.11 | 10.44 ± 0.13 | 10.03 ± 0.08** | 9.65 ± 0.14** |
| Reticulocytes (10 ³ /μL) | 70 ± 43 | 93 ± 45 | 108 ± 135 | 80 ± 24 | 75 ± 27 | 102 ± 115 |
| Howell-Jolly bodies (% erythrocytes) | 0.1 ± 0.0 | 0.0 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.2 ± 0.0 |
| Mean cell volume (fL) | 48.0 ± 0.3 | 48.4 ± 0.2 | 48.1 ± 0.2 | 48.2 ± 0.4 | 47.9 ± 0.3 | 48.0 ± 0.4 |
| Mean cell hemoglobin (pg) | 16.1 ± 0.1 | 16.2 ± 0.1 | 16.0 ± 0.1 | 16.0 ± 0.1 | 15.9 ± 0.1 | 16.0 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | 33.5 ± 0.1 | 33.4 ± 0.2 | 33.3 ± 0.2 | 33.1 ± 0.2 | 33.4 ± 0.2 | 33.3 ± 0.1 |
| Platelets (10 ³ /μL) | 961.6 ± 16.8 | 932.9 ± 20.3 | 969.7 ± 25.4 | 993.5 ± 18.9 | 996.2 ± 18.3 | 1,017.3 ± 27.7 |
| Leukocytes (10 ³ /μL) | 4.15 ± 0.44 | 3.36 ± 0.29 | 3.16 ± 0.28 | 3.71 ± 0.31 | 2.89 ± 0.34 | 3.16 ± 0.39 |
| Segmented neutrophils (10 ³ /μL) | 0.54 ± 0.08 | 0.49 ± 0.08 | 0.51 ± 0.12 | 0.67 ± 0.12 | 0.65 ± 0.12 | 0.82 ± 0.13 |
| Lymphocytes (10 ³ /μL) | 3.52 ± 0.38 | 2.77 ± 0.25 | 2.57 ± 0.27 | 2.93 ± 0.26 | 2.14 ± 0.28** | 2.25 ± 0.31** |
| Monocytes (10 ³ /μL) | 0.07 ± 0.02 | 0.07 ± 0.02 | 0.04 ± 0.01 | 0.08 ± 0.01 | 0.07 ± 0.01 | 0.05 ± 0.01 |
| Eosinophils (10 ³ /μL) | 0.02 ± 0.01 | 0.04 ± 0.02 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.04 ± 0.02 |
| Urinalysis | | | | | | |
| n | 10 | 10 | 9 | 10 | 10 | 10 |
| Fluoride (μg/16 hr) | 1.35 ± 0.10 ^b | 19.68 ± 3.15** ^c | 18.06 ± 4.66** ^d | 25.94 ± 3.62** | 56.48 ± 11.66** | 66.20 ± 6.15** |
| Glucose (mg/16 hr) | 0.16 ± 0.01 | 0.13 ± 0.02 | 0.09 ± 0.02 | 0.13 ± 0.01 | 0.12 ± 0.03 | 0.16 ± 0.02 |
| Protein (mg/16 hr) | 1 ± 0 | 1 ± 0 | 1 ± 0 | 1 ± 0 | 2 ± 0 | 2 ± 0 |
| Volume (mL/16 hr) | 2.4 ± 0.5 | 1.2 ± 0.3 | 0.8 ± 0.2 | 2.0 ± 0.4 | 5.9 ± 1.2 | 5.6 ± 0.8 |
| Specific gravity | 1.027 ± 0.002 | 1.038 ± 0.004 ^b | 1.039 ± 0.004 | 1.025 ± 0.002 | 1.013 ± 0.002** | 1.015 ± 0.003** |

TABLE G5
Hematology and Urinalysis Data for Mice in the 13-Week Inhalation Study of Tetrafluoroethylene (continued)

| | 0 ppm | 312 ppm | 625 ppm | 1,250 ppm | 2,500 ppm | 5,000 ppm |
|--|--------------------------|---------------|----------------|---------------|-----------------------------|-----------------|
| Female | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematology | | | | | | |
| Hematocrit (%) | 49.1 ± 0.5 | 48.8 ± 0.3 | 48.4 ± 0.3 | 49.5 ± 0.3 | 48.3 ± 0.5 | 47.1 ± 0.5** |
| Hemoglobin (g/dL) | 16.4 ± 0.2 | 16.4 ± 0.1 | 16.3 ± 0.1 | 16.3 ± 0.1 | 16.2 ± 0.2 | 15.8 ± 0.1** |
| Erythrocytes (10 ⁶ /μL) | 10.20 ± 0.09 | 10.27 ± 0.07 | 10.16 ± 0.07 | 10.29 ± 0.06 | 10.17 ± 0.10 | 9.93 ± 0.11 |
| Reticulocytes (10 ³ /μL) | 111 ± 40 | 113 ± 39 | 90 ± 42 | 108 ± 61 | 132 ± 51 | 117 ± 52 |
| Howell-Jolly bodies (% erythrocytes) | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 |
| Mean cell volume (fL) | 48.1 ± 0.2 | 47.6 ± 0.3 | 47.7 ± 0.3 | 48.1 ± 0.3 | 47.6 ± 0.2 | 47.4 ± 0.3 |
| Mean cell hemoglobin (pg) | 16.1 ± 0.1 | 16.0 ± 0.1 | 16.0 ± 0.1 | 15.9 ± 0.1 | 15.9 ± 0.1 | 15.9 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | 33.4 ± 0.2 | 33.7 ± 0.1 | 33.6 ± 0.1 | 33.0 ± 0.2 | 33.5 ± 0.2 | 33.6 ± 0.1 |
| Platelets (10 ³ /μL) | 899.1 ± 24.7 | 893.5 ± 14.5 | 901.0 ± 17.2 | 868.1 ± 17.4 | 911.2 ± 20.2 | 945.1 ± 21.2 |
| Leukocytes (10 ³ /μL) | 3.80 ± 0.25 | 3.18 ± 0.14* | 3.17 ± 0.23* | 3.36 ± 0.26 | 2.62 ± 0.30** | 2.97 ± 0.21** |
| Segmented neutrophils (10 ³ /μL) | 0.59 ± 0.08 | 0.50 ± 0.06 | 0.30 ± 0.03* | 0.44 ± 0.05 | 0.39 ± 0.09 | 0.42 ± 0.07 |
| Lymphocytes (10 ³ /μL) | 3.11 ± 0.22 | 2.62 ± 0.11 | 2.78 ± 0.24 | 2.76 ± 0.21 | 2.15 ± 0.21** | 2.41 ± 0.19** |
| Monocytes (10 ³ /μL) | 0.03 ± 0.02 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.07 ± 0.02 | 0.06 ± 0.02 | 0.10 ± 0.03 |
| Eosinophils (10 ³ /μL) | 0.07 ± 0.02 | 0.03 ± 0.01 | 0.05 ± 0.02 | 0.08 ± 0.02 | 0.02 ± 0.01 | 0.04 ± 0.01 |
| Urinalysis | | | | | | |
| Fluoride (μg/16 hr) | 1.01 ± 0.07 ^b | 7.79 ± 2.20** | 12.05 ± 2.73** | 8.74 ± 1.38** | 19.34 ± 3.65** ^b | 23.94 ± 1.72** |
| Glucose (mg/16 hr) | 0.12 ± 0.02 | 0.13 ± 0.02 | 0.12 ± 0.02 | 0.13 ± 0.02 | 0.18 ± 0.03 | 0.22 ± 0.02* |
| Protein (mg/16 hr) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 1 ± 0 |
| Volume (mL/16 hr) | 1.1 ± 0.2 | 1.2 ± 0.3 | 1.0 ± 0.1 | 1.1 ± 0.2 | 3.9 ± 0.8* | 6.4 ± 1.0** |
| Specific gravity | 1.025 ± 0.003 | 1.025 ± 0.003 | 1.027 ± 0.003 | 1.022 ± 0.002 | 1.012 ± 0.002** | 1.008 ± 0.001** |

* Statistically different (P<0.05) from the control group by Dunn's or Shirley's test

** P<0.01

^a Mean ± standard error; statistical tests were performed on unrounded data.

^b n=9

^c n=7

^d n=5

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS
AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1 **Summary of Reproductive Tissue Evaluations**
for Male Rats in the 13-Week Inhalation Study of Tetrafluoroethylene **274**

TABLE H2 **Summary of Estrous Cycle Characterization**
for Female Rats in the 13-Week Inhalation Study of Tetrafluoroethylene **274**

TABLE H3 **Summary of Reproductive Tissue Evaluations**
for Male Mice in the 13-Week Inhalation Study of Tetrafluoroethylene **275**

TABLE H4 **Summary of Estrous Cycle Characterization**
for Female Mice in the 13-Week Inhalation Study of Tetrafluoroethylene **275**

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 13-Week Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 1,250 ppm | 5,000 ppm |
|---|---------------|---------------|---------------|---------------|
| n | 10 | 10 | 10 | 10 |
| Weights (g) | | | | |
| Necropsy body wt | 329 ± 5 | 343 ± 5 | 333 ± 4 | 301 ± 5** |
| R. cauda | 0.170 ± 0.004 | 0.175 ± 0.004 | 0.161 ± 0.004 | 0.161 ± 0.005 |
| R. epididymis | 0.472 ± 0.008 | 0.479 ± 0.010 | 0.469 ± 0.010 | 0.477 ± 0.009 |
| R. testis | 1.384 ± 0.023 | 1.412 ± 0.029 | 1.375 ± 0.031 | 1.417 ± 0.020 |
| Epididymal spermatozoal parameters | | | | |
| Abnormal sperm (%) | 0.680 ± 0.085 | 0.480 ± 0.080 | 0.640 ± 0.088 | 0.600 ± 0.107 |
| Motility (%) | 82.22 ± 1.29 | 80.60 ± 1.63 | 79.42 ± 1.96 | 80.21 ± 0.76 |
| Concentration (10 ⁶ /g cauda epididymal tissue) | 452 ± 25 | 445 ± 21 | 408 ± 34 | 426 ± 20 |

** Significantly different ($P \leq 0.01$) from the control group by Williams' test

^a Data are presented as mean ± standard error. Differences from the control group were not significant by Dunnett's test (organ weights) or Dunn's test (epididymal spermatozoal parameters).

TABLE H2
Summary of Estrous Cycle Characterization for Female Rats in the 13-Week Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 1,250 ppm | 5,000 ppm |
|-----------------------------|-------------|-------------|--------------------------|--------------------------|
| n | 10 | 10 | 10 | 10 |
| Necropsy body wt (g) | 189 ± 5 | 198 ± 3 | 194 ± 4 | 173 ± 4* |
| Estrous cycle length (days) | 4.70 ± 0.21 | 5.10 ± 0.18 | 5.00 ± 0.27 ^b | 5.43 ± 0.43 ^c |
| Estrous stage (% of cycle) | | | | |
| Diestrus | 38.6 | 42.9 | 35.7 | 51.4 |
| Proestrus 14.3 | 12.9 | 11.4 | 21.4 | |
| Estrus | 21.4 | 27.1 | 27.1 | 14.3 |
| Metestrus 24.3 | 17.1 | 25.7 | 12.9 | |
| Unclear diagnosis | 1.4 | 0.0 | 0.0 | 0.0 |

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

^a Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.

^b Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.

^c Estrous cycle was longer than 12 days or unclear in 3 of 10 animals.

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 13-Week Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 1,250 ppm | 5,000 ppm |
|---|----------------------------|---------------|---------------|----------------|
| n | 10 | 10 | 10 | 10 |
| Weights (g) | | | | |
| Necropsy body wt | 36.2 ± 0.7 | 35.3 ± 0.5 | 34.6 ± 0.5* | 32.1 ± 0.5** |
| R. cauda | 0.016 ± 0.001 | 0.017 ± 0.001 | 0.016 ± 0.000 | 0.014 ± 0.000* |
| R. epididymis | 0.049 ± 0.002 | 0.048 ± 0.001 | 0.047 ± 0.001 | 0.045 ± 0.002 |
| R. testis | 0.117 ± 0.003 | 0.117 ± 0.002 | 0.118 ± 0.001 | 0.112 ± 0.003 |
| Epididymal spermatozoal parameters | | | | |
| Abnormal sperm (%) | 1.100 ± 0.141 ^b | 0.920 ± 0.108 | 1.360 ± 0.168 | 0.860 ± 0.116 |
| Motility (%) | 75.36 ± 4.15 | 73.56 ± 2.02 | 70.08 ± 1.26* | 69.89 ± 2.60 |
| Concentration (10 ⁶ /g cauda epididymal tissue) | 976 ± 134 ^b | 1,010 ± 62 | 921 ± 72 | 947 ± 101 |

* Significantly different ($P \leq 0.05$) from the control group by Williams' test (body weights), Dunnett's test (organ weights), or Dunn's test (epididymal spermatozoal parameters)

** Significantly different ($P \leq 0.01$) from the control group by Williams' test

^a Data are presented as mean ± standard error.

^b n=8

TABLE H4
Summary of Estrous Cycle Characterization for Female Mice in the 13-Week Inhalation Study of Tetrafluoroethylene^a

| | 0 ppm | 312 ppm | 1,250 ppm | 5,000 ppm |
|-----------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| n | 10 | 10 | 10 | 10 |
| Necropsy body wt (g) | 31.5 ± 0.7 | 30.8 ± 0.8 | 29.8 ± 0.6 | 29.2 ± 0.5 |
| Estrous cycle length (days) | 4.33 ± 0.24 ^b | 4.88 ± 0.23 ^c | 4.56 ± 0.18 ^b | 5.22 ± 0.28 ^b |
| Estrous stage (% of cycle) | | | | |
| Diestrus | 34.3 | 42.9 | 34.3 | 31.4 |
| Proestrus 18.6 | 11.4 | 22.9 | 20.0 | |
| Estrus | 28.6 | 30.0 | 22.9 | 27.1 |
| Metestrus 18.6 | 15.7 | 20.0 | 21.4 | |

^a Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for body weights were not significant by Dunnett's test. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.

^b Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

^c Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.

APPENDIX I

CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

| | |
|--|-----|
| PROCUREMENT AND CHARACTERIZATION OF TETRAFLUOROETHYLENE | 278 |
| VAPOR GENERATION AND EXPOSURE SYSTEM | 279 |
| VAPOR CONCENTRATION MONITORING | 279 |
| CHAMBER ATMOSPHERE CHARACTERIZATION | 279 |
| FIGURE I1 Infrared Absorption Spectrum of Tetrafluoroethylene | 281 |
| FIGURE I2 Schematic of the Generation and Delivery System | 282 |
| FIGURE I3 Schematic of the Exposure Chambers | 283 |
| FIGURE I4 16-Day and 13-Week Inhalation Suite | 284 |
| FIGURE I5 2-Year Inhalation Suite | 285 |
| FIGURE I6 Schematic of the Concentration Monitoring System | 286 |
| TABLE I1 Summary of Chamber Concentrations in the 16-Day Inhalation Studies of Tetrafluoroethylene | 287 |
| TABLE I2 Summary of Chamber Concentrations in the 13-Week Inhalation Studies of Tetrafluoroethylene | 287 |
| TABLE I3 Summary of Chamber Concentrations in the 2-Year Inhalation Studies of Tetrafluoroethylene | 288 |
| FIGURE I7 Monthly Mean Concentrations and Standard Deviations in the 156 ppm Tetrafluoroethylene Male Rat Exposure Chamber for the 2-Year Study | 289 |
| FIGURE I8 Monthly Mean Concentrations and Standard Deviations in the 312 ppm Tetrafluoroethylene Male Rat Exposure Chamber for the 2-Year Study | 290 |
| FIGURE I9 Monthly Mean Concentrations and Standard Deviations in the 625 ppm Tetrafluoroethylene Male Rat Exposure Chamber for the 2-Year Study | 291 |
| FIGURE I10 Monthly Mean Concentrations and Standard Deviations in the 312 ppm Tetrafluoroethylene Female Rat Exposure Chamber for the 2-Year Study | 292 |
| FIGURE I11 Monthly Mean Concentrations and Standard Deviations in the 625 ppm Tetrafluoroethylene Female Rat Exposure Chamber for the 2-Year Study | 293 |
| FIGURE I12 Monthly Mean Concentrations and Standard Deviations in the 1,250 ppm Tetrafluoroethylene Female Rat Exposure Chamber for the 2-Year Study | 294 |
| FIGURE I13 Monthly Mean Concentrations and Standard Deviations in the 312 ppm Tetrafluoroethylene Mouse Exposure Chamber for the 2-Year Study | 295 |
| FIGURE I14 Monthly Mean Concentrations and Standard Deviations in the 625 ppm Tetrafluoroethylene Mouse Exposure Chamber for the 2-Year Study | 296 |
| FIGURE I15 Monthly Mean Concentrations and Standard Deviations in the 1,250 ppm Tetrafluoroethylene Mouse Exposure Chamber for the 2-Year Study | 297 |

CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

PROCUREMENT AND CHARACTERIZATION OF TETRAFLUOROETHYLENE

Tetrafluoroethylene was obtained from SCM Specialty Chemicals (Gainesville, FL) in one lot (10271), which was used for the identity and purity analyses conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). Tetrafluoroethylene was also obtained from ICI Americas, Inc. (Bayonne, NJ), in five lots. The study laboratory assigned a lot number to each shipment of as many as 12 cylinders. Lot 12017-8 was used during the 16-day studies and during most of the 13-week studies; lot 12017-77 was used during the remainder of the 13-week studies; and lots 12438-6, 12438-79, and 12438-92 were used during the 2-year studies. Identity and purity analyses were also conducted by the study laboratory on each lot used during the studies. Reports on analyses performed in support of the tetrafluoroethylene studies are on file at the National Institute of Environmental Health Sciences.

Each lot of the chemical, a colorless gas, was identified as tetrafluoroethylene by the analytical chemistry or study laboratory using infrared spectroscopy. The infrared spectrum for each lot was consistent with that expected for the structure of tetrafluoroethylene (Figure II).

The purity of lot 10271 was determined by the analytical chemistry laboratory using gas chromatography. The purity of lots 12017-8, 12017-77, 12438-6, 12438-79, and 12438-92 was determined by the study laboratory using gas chromatography. Gas chromatography of each lot was conducted using a flame ionization detector with a nitrogen carrier gas at a flow rate of 70 mL/minute, a 60/80 Carboxen B/1% glass column, and an oven temperature program of 35 ° C for 5 minutes, then 35 ° to 200 ° C at 10 ° C per minute.

Gas chromatography of lot 10271 indicated one major peak and one impurity with an area greater than or equal to 0.1% relative to the major peak. In addition, analysis for *d*-limonene, which is added to tetrafluoroethylene as a stabilizer, was conducted by the analytical chemistry laboratory using gas chromatography with the same system as that used for the purity analyses but with a 10% SP-2100 on 100/120 Supelcoport glass column and an oven temperature program of 50 ° C for 5 minutes, then 50 ° to 250 ° C at 10 ° C per minute. The sample contained 0.03% ± 0.00% *d*-limonene. Gas chromatography conducted by the study laboratory for each cylinder of lots 12017-8 and 12017-77 indicated that perfluorocyclobutane, the most abundant dimer produced during tetrafluoroethylene decomposition, was present at concentrations less than or equal to 462 ppm and 2,604 ppm for the 16-day and 13-week studies, respectively. For lots 12438-6, 12438-79, and 12438-92, gas chromatography indicated peaks for perfluorocyclobutane with areas less than or equal to 1.21% relative to the major peak. Gas chromatography conducted by the study laboratory for each cylinder of lots 12017-8 and 12017-77 indicated that *d*-limonene was present at concentrations less than or equal to 2,837 ppm for the 13-week studies. During the 2-year studies, gas chromatography of lots 12438-6, 12438-79, and 12438-92 indicated peaks for *d*-limonene with areas less than or equal to 0.56% relative to the major peak. The manufacturer indicated that trifluoroethylene, methylene fluoride, vinyl fluoride, and vinylidene fluoride were also present as impurities at concentrations less than or equal to 1.7 ppm. The overall purities of lots 12017-8, 12017-77, and 12438-92 were determined to be greater than 99%. The overall purities of lots 12438-6 and 12438-79 were determined to be greater than 98%.

The manufacturer indicated that tetrafluoroethylene would be stable for up to 1 year when stored in the original containers at room temperature. To ensure stability, the bulk chemical was stored in the original metal cylinders at 10 ° to 22 ° C. Stability was monitored during the 13-week and 2-year studies using the same gas chromatography system used for the purity analyses. The relative concentrations of perfluorocyclobutane were expected to increase due to the slow dimerization of tetrafluoroethylene, and therefore the gas cylinders were monitored for this dimer as well as for *d*-limonene during the 2-year studies. Two cylinders from lot 12438-6 contained higher concentrations of these compounds than canisters from the same lot that were analyzed earlier in the studies. One of these two cylinders, which contained 1.21% perfluorocyclobutane and 0.56% *d*-limonene, was replaced immediately after gas chromatographic analysis was performed; it is not known if the dimer formed in the canister due to degradation of tetrafluoroethylene or was present as an impurity at receipt. Stability was generally considered acceptable throughout the studies.

VAPOR GENERATION AND EXPOSURE SYSTEM

A diagram of the tetrafluoroethylene generation and delivery system is shown in Figure I2. Because tetrafluoroethylene is a gas at room temperature, the system incorporated gas distribution under regulated pressure with individual adjustment and monitoring of the chemical flow rate to each chamber. Tetrafluoroethylene was taken directly from the cylinder in which it was shipped. The output pressure from the cylinder was regulated to 20 psi with a two-stage regulator. The gas was passed through a main exposure pneumatic valve, a check valve, and a filter to a manifold of five (16-day and 13-week studies) or six (2-year studies) flow meters with integral metering valves. The gas was metered to each exposure chamber through these flow meters, which were monitored by a pressure gauge at the manifold. A three-way solenoid valve was located in the gas delivery line to each exposure chamber. This valve and a nitrogen inlet valve just downstream from the main exposure valve allowed the system to be purged with nitrogen at the end of each exposure period. A sample line was included downstream from each gas cylinder. All equipment that came in contact with the tetrafluoroethylene gas was stainless steel, polytetrafluoroethylene, Viton® (E.I. DuPont de Nemours, Wilmington, DE), or glass.

Stainless-steel chambers designed at Battelle Pacific Northwest Laboratories were used for all studies (Figure I3). The total volume of each chamber was 2.3 m³; the active mixing volume of each chamber was 1.7 m³. The chamber was designed so that uniform aerosol or vapor concentrations could be maintained throughout the chamber when the catch pans were in position, provided the aerosol or vapor was uniformly mixed with dilution air before entering the chamber. A diagram of the 16-day and 13-week exposure suite is shown in Figure I4, and a diagram of the 2-year exposure suite is shown in Figure I5.

VAPOR CONCENTRATION MONITORING

A diagram of the tetrafluoroethylene concentration monitoring system used in all studies is shown in Figure I6. The concentrations of tetrafluoroethylene were monitored using an on-line gas chromatograph with a flame ionization detector, an 80/100 Porapak Q nickel column, and a nitrogen carrier gas at a flow rate of 22 mL/minute. Samples were drawn and analyzed from each exposure chamber, the control chamber, the exposure suite, and an on-line standard using an automatic 8-port (16-day and 13-week studies) or 12-port (2-year studies) sample valve. Certified standards of tetrafluoroethylene in nitrogen (Byrne Specialty Gases, Inc., Seattle, WA; MG Industries Scientific Gases, Los Angeles, CA; Phoenix Distributors, Inc., Los Angeles, CA) were used to perform the absolute calibration of the on-line gas chromatograph and to check instrument drift throughout the exposure periods. Summaries of the chamber concentrations during the studies are presented in Tables I1 through I3. The monthly mean exposure concentrations for the 2-year studies are presented in Figures I7 through I15.

CHAMBER ATMOSPHERE CHARACTERIZATION

The times for the exposure concentration to build up to 90% of the final exposure concentration (T_{90}) and to decay to 10% of the exposure concentration (T_{10}) were measured in all studies with and without animals present. During the 16-day studies, T_{90} was approximately 12 minutes and T_{10} was approximately 9 minutes with and without animals in the chambers. During the 13-week studies, T_{90} was 11 minutes with animals in the chambers and ranged from 11 to 14 minutes without animals; T_{10} ranged from 9 to 11 minutes with and without animals in the chambers. During the 2-year studies, T_{90} ranged from 8 to 10 minutes without animals in the chambers and from 10 to 16 minutes with animals present; T_{10} ranged from 7 to 9 minutes without animals and from 9 to 12 minutes with animals. The T_{90} value used for all studies was 12 minutes.

Uniformity of tetrafluoroethylene concentration in the exposure chambers was measured prior to the start of all studies, once during the 16-day and 13-week studies, and approximately every 3 months during the 2-year studies. The concentration was measured with and without animals present using the on-line gas chromatograph with the automatic 8-port (16-day and 13-week studies) or 12-port (2-year studies) sample valve disabled to allow continuous monitoring from a single input line. Uniformity of exposure concentrations in all chambers was acceptable.

The persistence of tetrafluoroethylene in the 1,250 ppm exposure chamber after shutting off the system was monitored without animals before the 2-year studies began and with animals present during the 2-year studies. The concentration of

tetrafluoroethylene in the exposure chamber fell rapidly to less than 1% of the beginning concentration within 20 minutes. Tetrafluoroethylene concentrations in the building exhaust and room air were also monitored during all studies.

Information supplied by the manufacturer indicated that all cylinders contained *d*-limonene as a stabilizer. *d*-Limonene and perfluorocyclobutane are less volatile than tetrafluoroethylene and concentrate in the cylinder as the tetrafluoroethylene is removed. Cylinder usage was regulated to minimize concentrations of these chemicals in the exposure chambers; a maximum of 80% of the tetrafluoroethylene in each cylinder was used. Gas chromatography of chamber samples did not indicate any impurities other than perfluorocyclobutane and *d*-limonene. Concurrent analysis of cylinder headspace and chamber samples for perfluorocyclobutane demonstrated that chamber concentrations of perfluorocyclobutane matched those in the cylinder headspace. The contents of selected used cylinders were analyzed for *d*-limonene and perfluorocyclobutane. The results indicated that tetrafluoroethylene was stable during generation and in the exposure chambers and that perfluorocyclobutane was not formed in significant quantities by the generation system. As expected, the headspace concentrations of *d*-limonene and perfluorocyclobutane in the cylinders increased as tetrafluoroethylene was removed.

Spectrum No. 332N Date 1/17/85 Sample Tetrafluoroethylene Lot No. 10271 Batch No. 01 Source Gas cell
Path 100 mm NaCl plates Solvent Neat Concentration --- Phase --- Task No. SB1433
Analyst T. Pederson and B. Heizelman Wavelength In Microns --- Project No. 8402-36

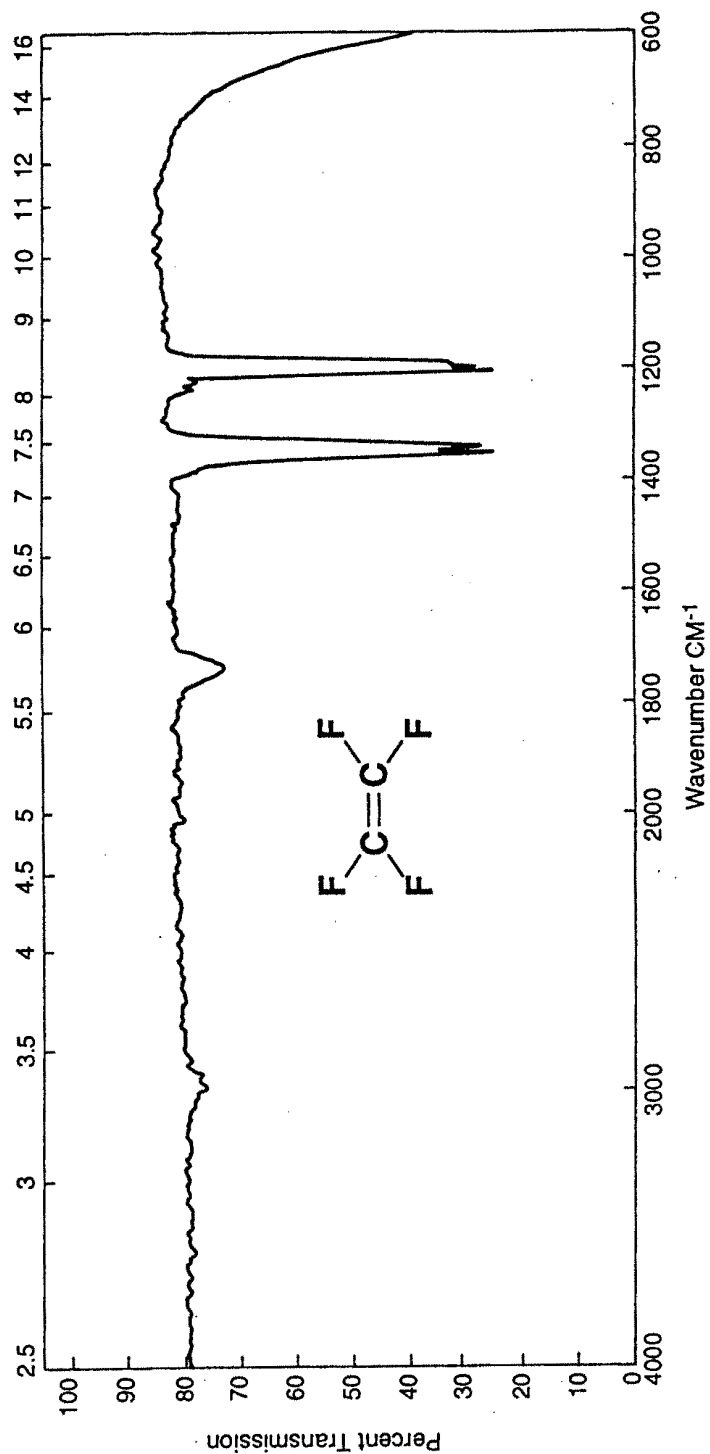


FIGURE II
Infrared Absorption Spectrum of Tetrafluoroethylene

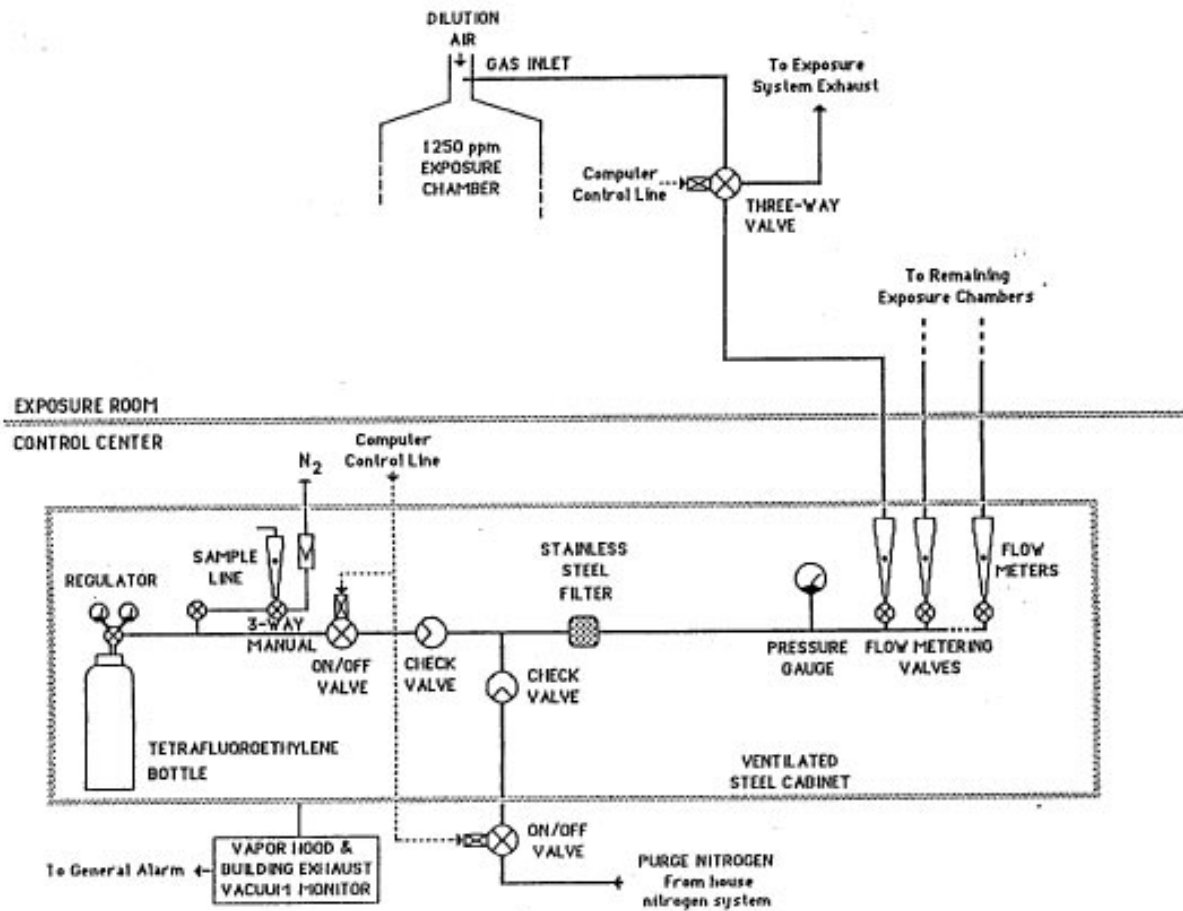
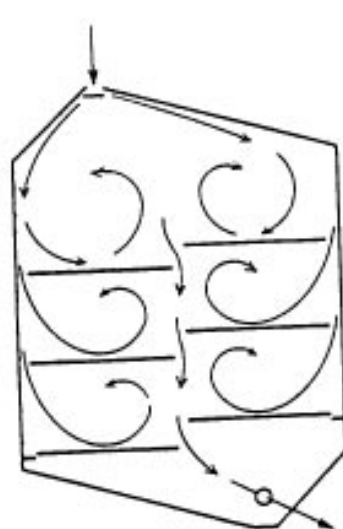
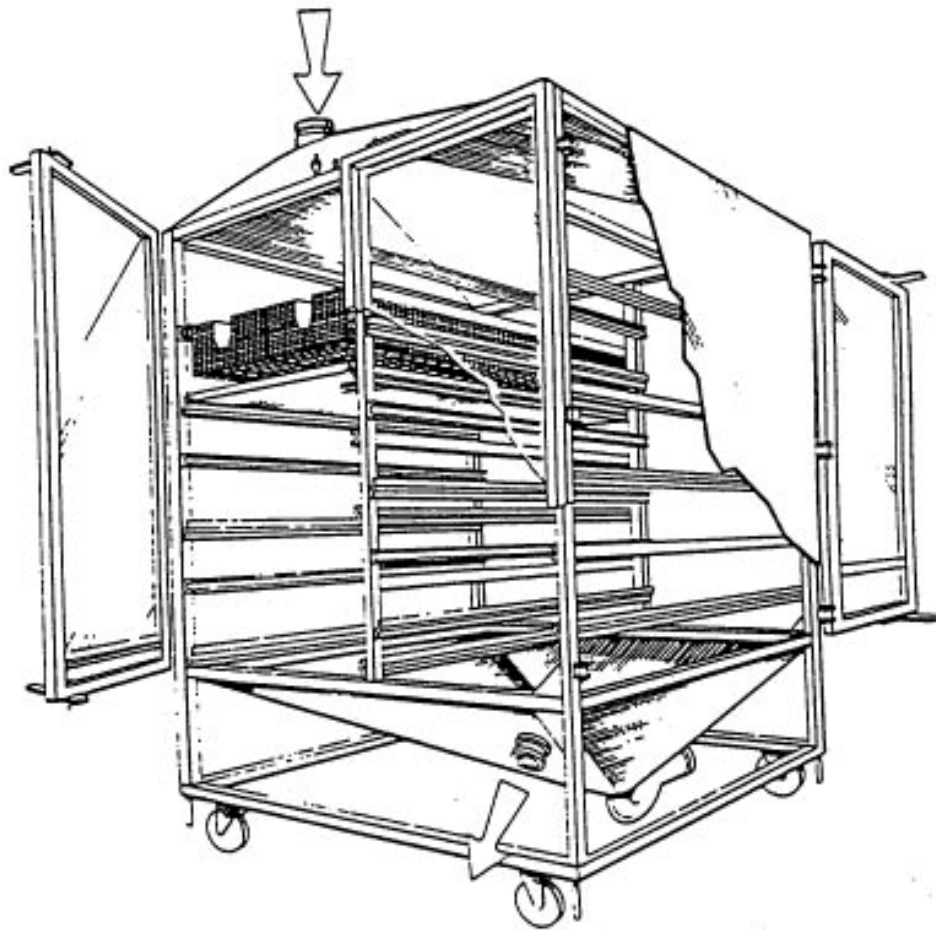
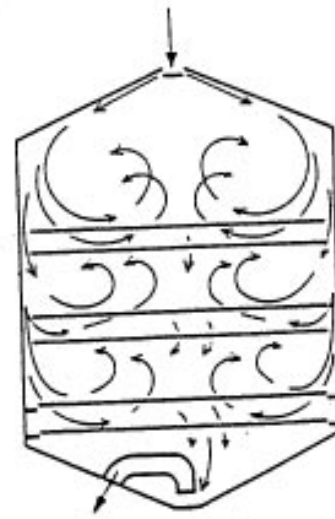


FIGURE I2
Schematic of the Generation and Delivery System



FRONT VIEW



SIDE VIEW

FIGURE I3
Schematic of the Exposure Chambers

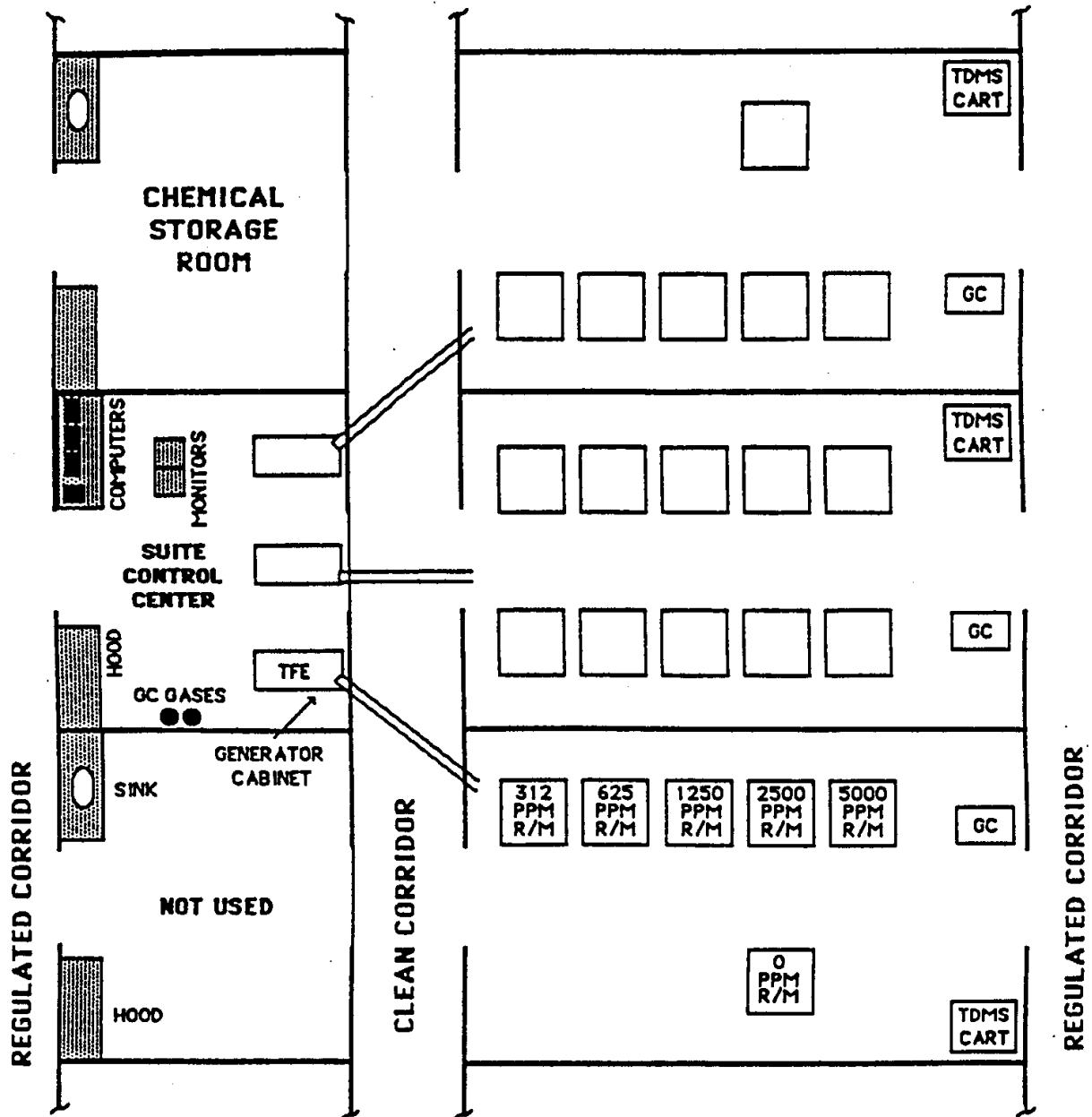


FIGURE I4
16-Day and 13-Week Inhalation Suite

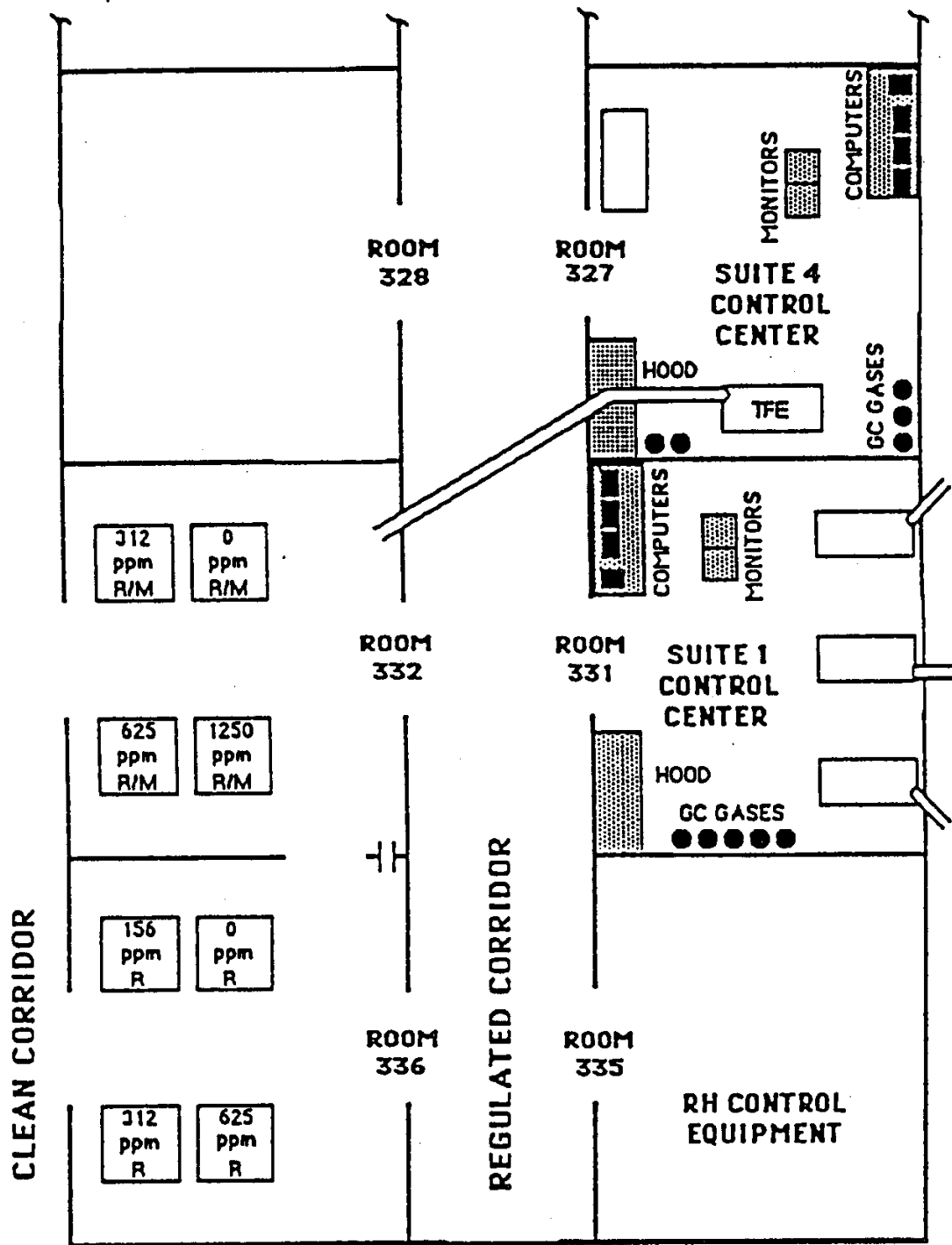


FIGURE I5
2-Year Inhalation Suite

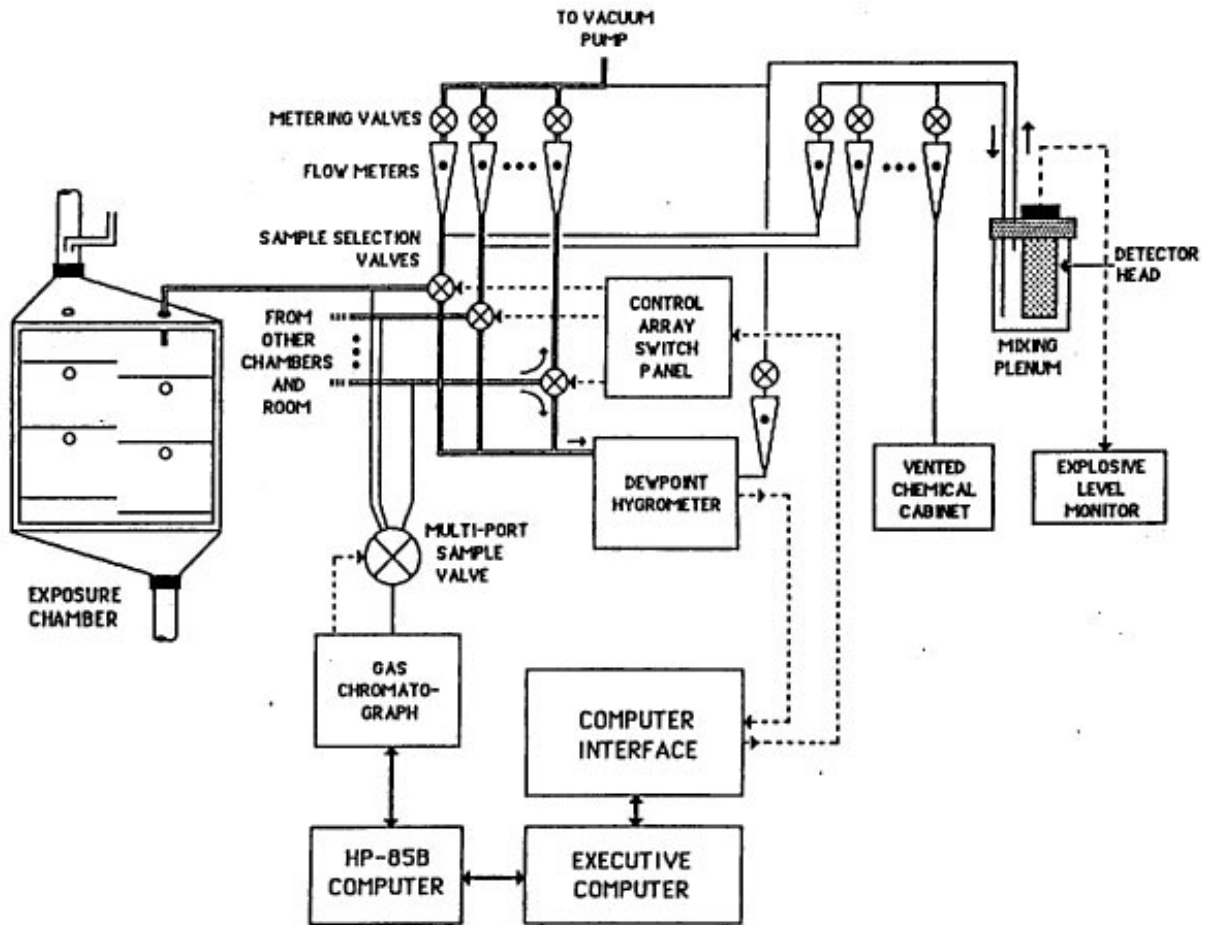


FIGURE I6
Schematic of the Concentration Monitoring System

TABLE I1
Summary of Chamber Concentrations in the 16-Day Inhalation Studies of Tetrafluoroethylene

| Target Concentration (ppm) | Total Number of Readings | Average Concentration ^a (ppm) |
|-------------------------------|--------------------------|---|
| Rat Chambers | | |
| 312 | 250 | 312 ± 6.21 |
| 625 | 250 | 621 ± 9.66 |
| 1,250 | 252 | 1,250 ± 19.2 |
| 2,500 | 253 | 2,500 ± 33.9 |
| 5,000 | 255 | 4,990 ± 6.19 |
| Mouse Chambers | | |
| 312 | 250 | 312 ± 5.60 |
| 625 | 249 | 622 ± 9.40 |
| 1,250 | 252 | 1,260 ± 19.2 |
| 2,500 | 253 | 2,500 ± 34.1 |
| 5,000 | 255 | 4,990 ± 59.4 |

^a Mean ± standard deviation

TABLE I2
Summary of Chamber Concentrations in the 13-Week Inhalation Studies of Tetrafluoroethylene

| Target Concentration (ppm) | Total Number of Readings | Average Concentration ^a (ppm) |
|-------------------------------|--------------------------|---|
| Rat Chambers | | |
| 312 | 1,107 | 314 ± 9.3 |
| 625 | 1,106 | 628 ± 16 |
| 1,250 | 1,146 | 1,260 ± 40 |
| 2,500 | 1,154 | 2,520 ± 77 |
| 5,000 | 1,149 | 5,050 ± 136 |
| Mouse Chambers | | |
| 312 | 1,104 | 314 ± 9.4 |
| 625 | 1,103 | 627 ± 16 |
| 1,250 | 1,145 | 1,260 ± 35 |
| 2,500 | 1,153 | 2,520 ± 67 |
| 5,000 | 1,147 | 5,050 ± 128 |

^a Mean ± standard deviation

TABLE I3
Summary of Chamber Concentrations in the 2-Year Inhalation Studies of Tetrafluoroethylene

| Target Concentration (ppm) | Total Number of Readings | Average Concentration ^a (ppm) |
|-------------------------------|--------------------------|---|
| Male Rat Chambers | | |
| 156 | 6,470 | 155 ± 7 |
| 312 | 6,523 | 312 ± 12 |
| 625 | 6,533 | 623 ± 24 |
| Female Rat Chambers | | |
| 312 | 6,106 | 312 ± 10 |
| 625 | 6,146 | 625 ± 20 |
| 1,250 | 6,154 | 1,250 ± 43 |
| Mouse Chambers | | |
| 312 | 5,577 | 311 ± 10 |
| 625 | 5,619 | 625 ± 19 |
| 1,250 | 5,633 | 1,250 ± 42 |

^a Mean ± standard deviation

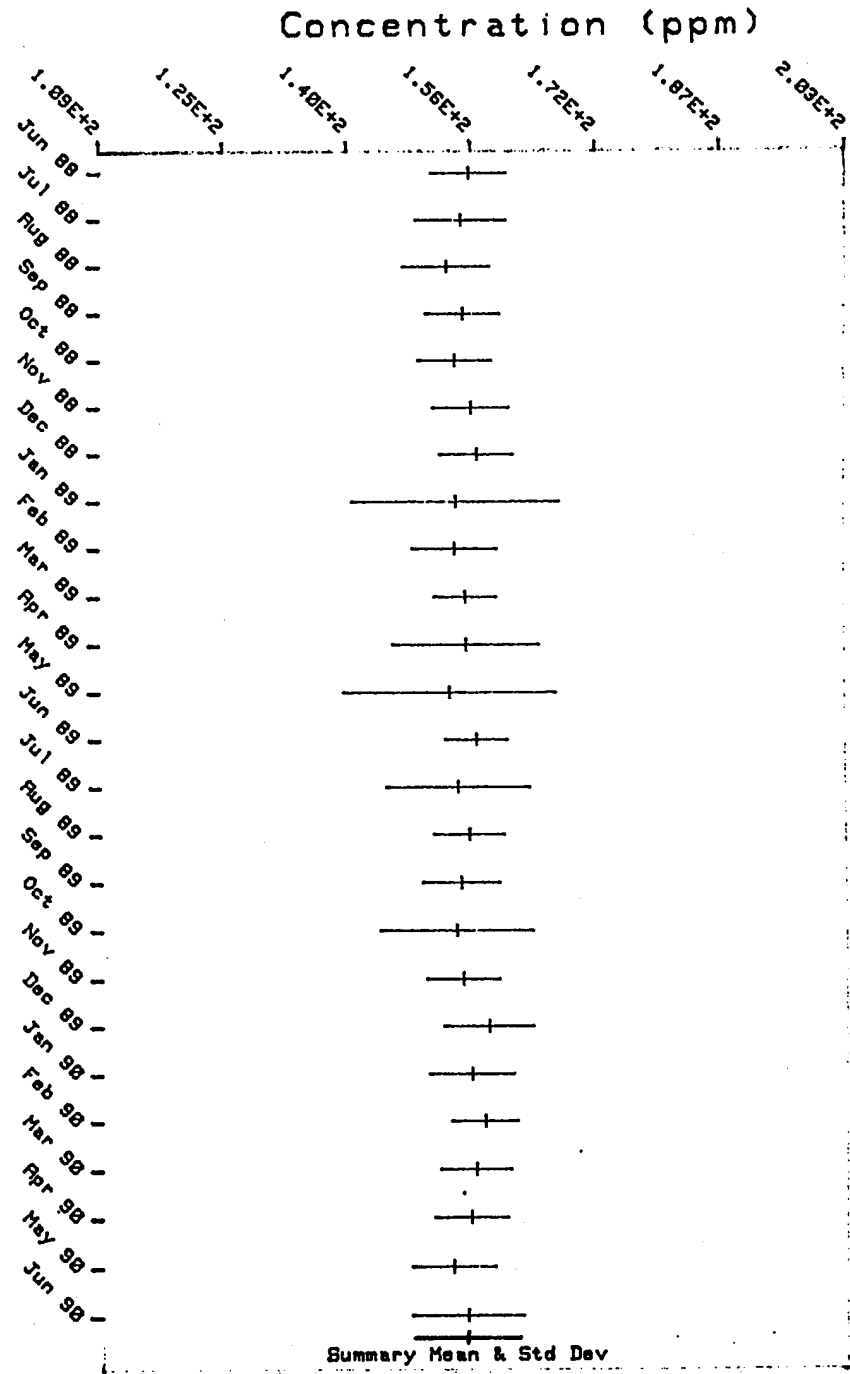


FIGURE I7
Monthly Mean Concentrations and Standard Deviations
in the 156 ppm Tetrafluoroethylene Male Rat Exposure Chamber
for the 2-Year Study

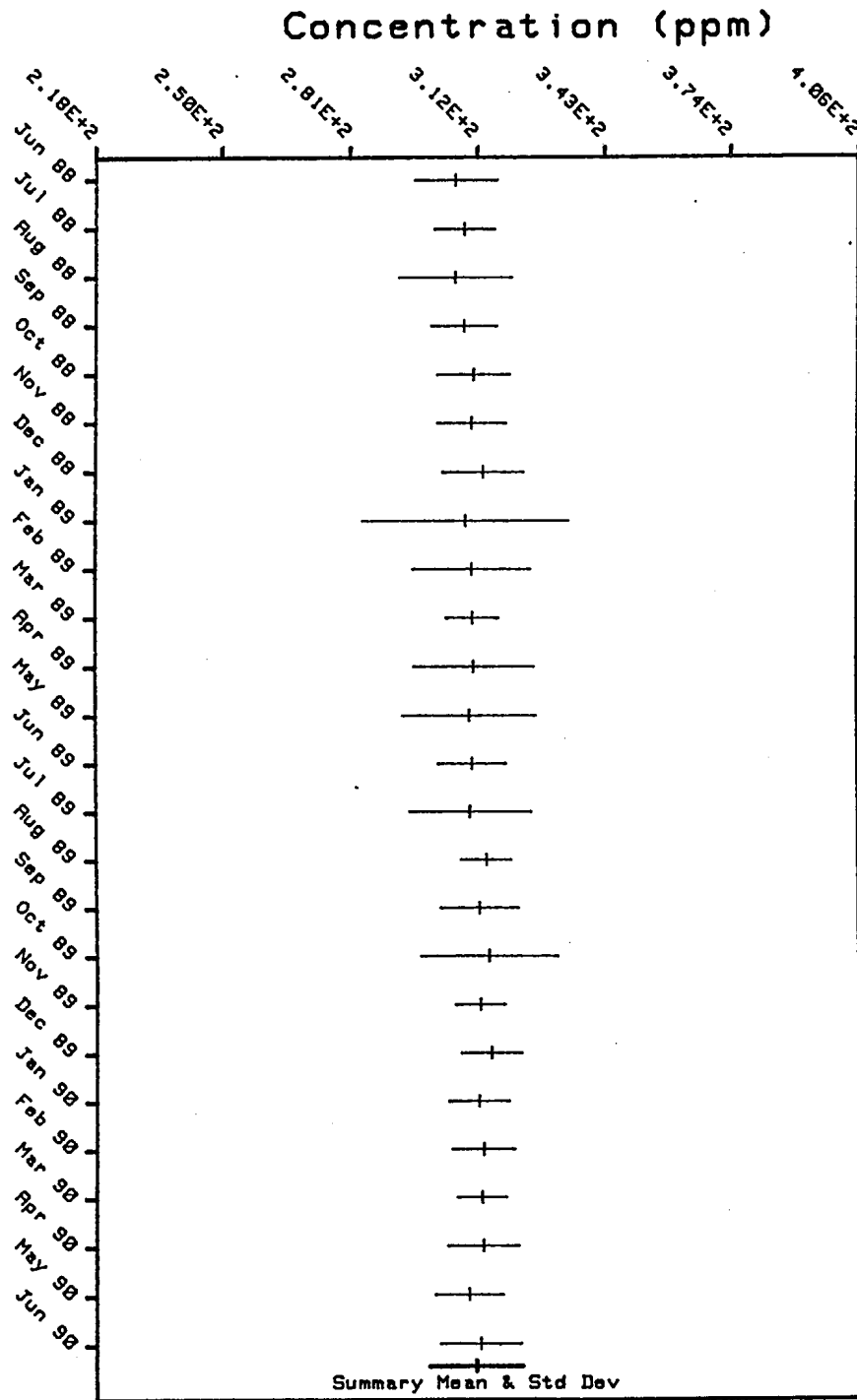


FIGURE I8
Monthly Mean Concentrations and Standard Deviations
in the 312 ppm Tetrafluoroethylene Male Rat Exposure Chamber
for the 2-Year Study

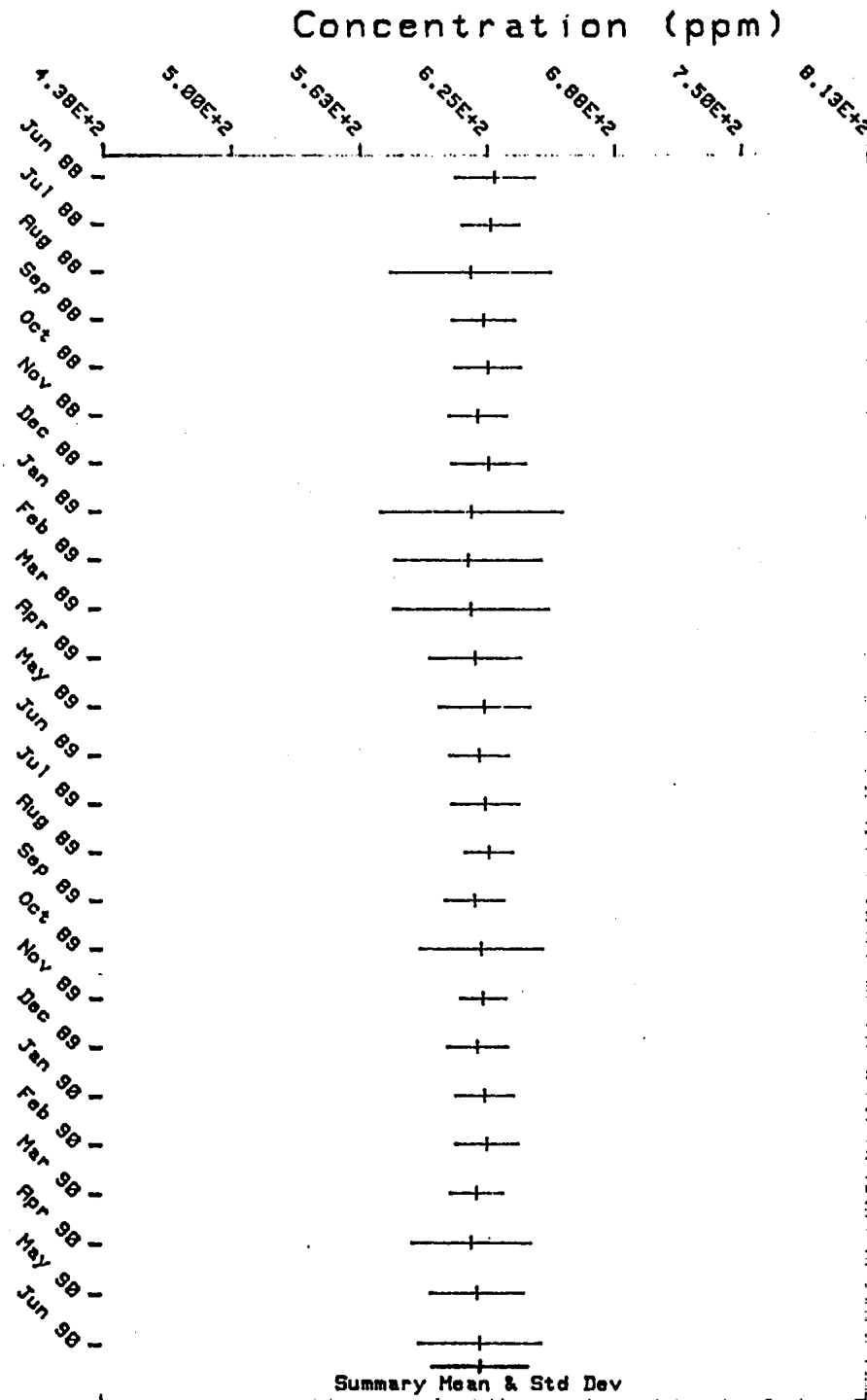


FIGURE I9
 Monthly Mean Concentrations and Standard Deviations
 in the 625 ppm Tetrafluoroethylene Male Rat Exposure Chamber
 for the 2-Year Study

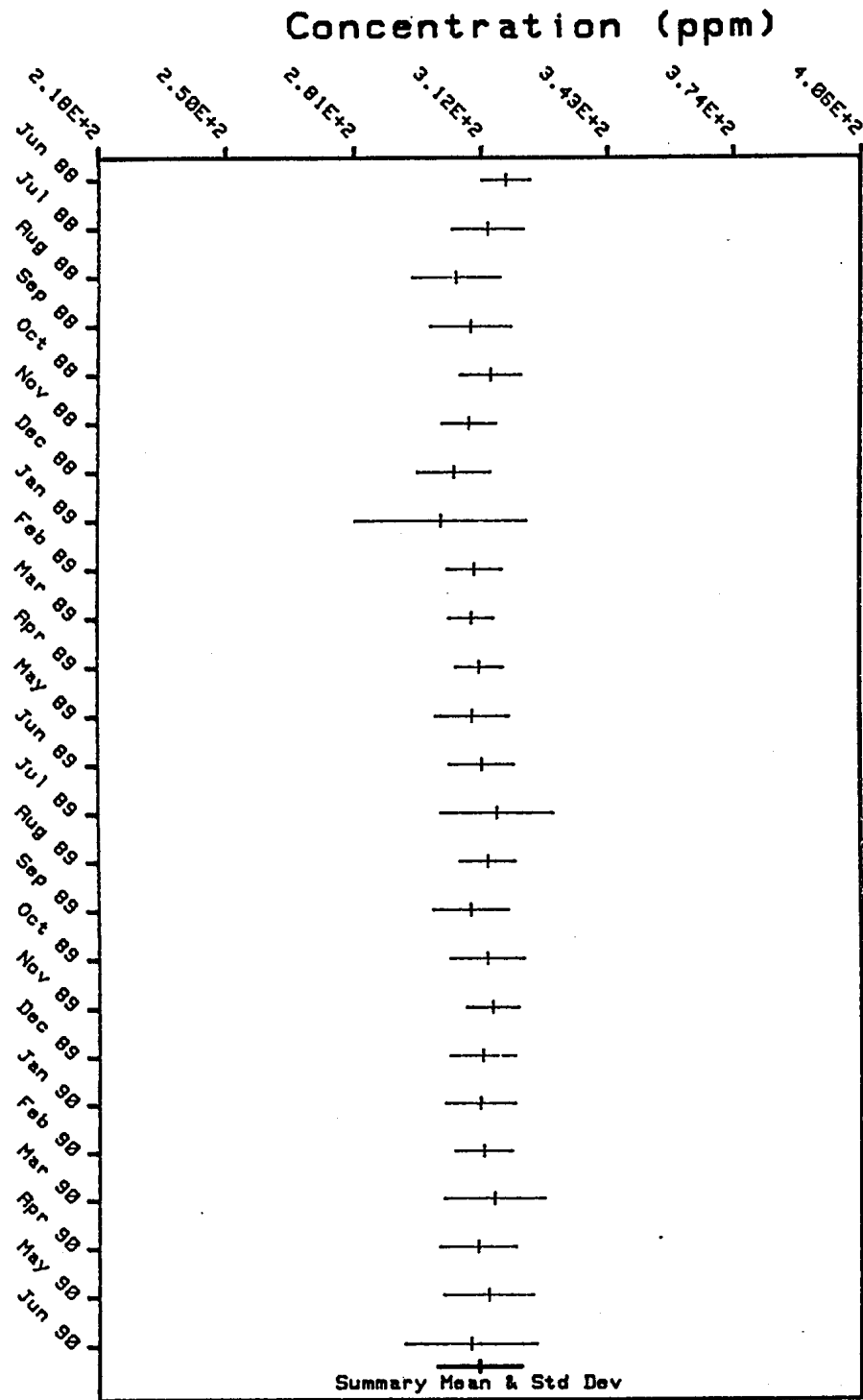


FIGURE I10
Monthly Mean Concentrations and Standard Deviations
in the 312 ppm Tetrafluoroethylene Female Rat Exposure Chamber
for the 2-Year Study

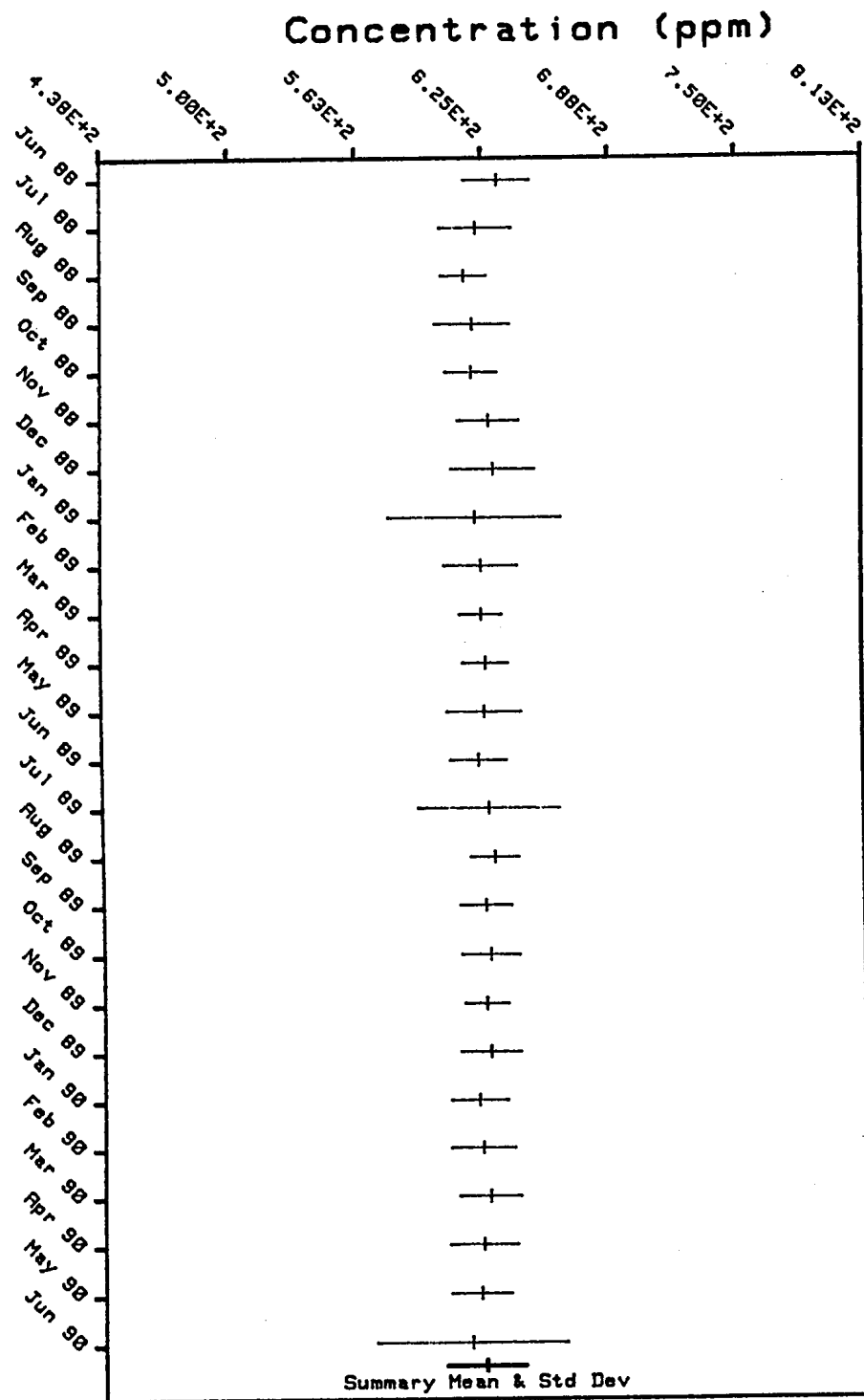


FIGURE I11
Monthly Mean Concentrations and Standard Deviations
in the 625 ppm Tetrafluoroethylene Female Rat Exposure Chamber
for the 2-Year Study

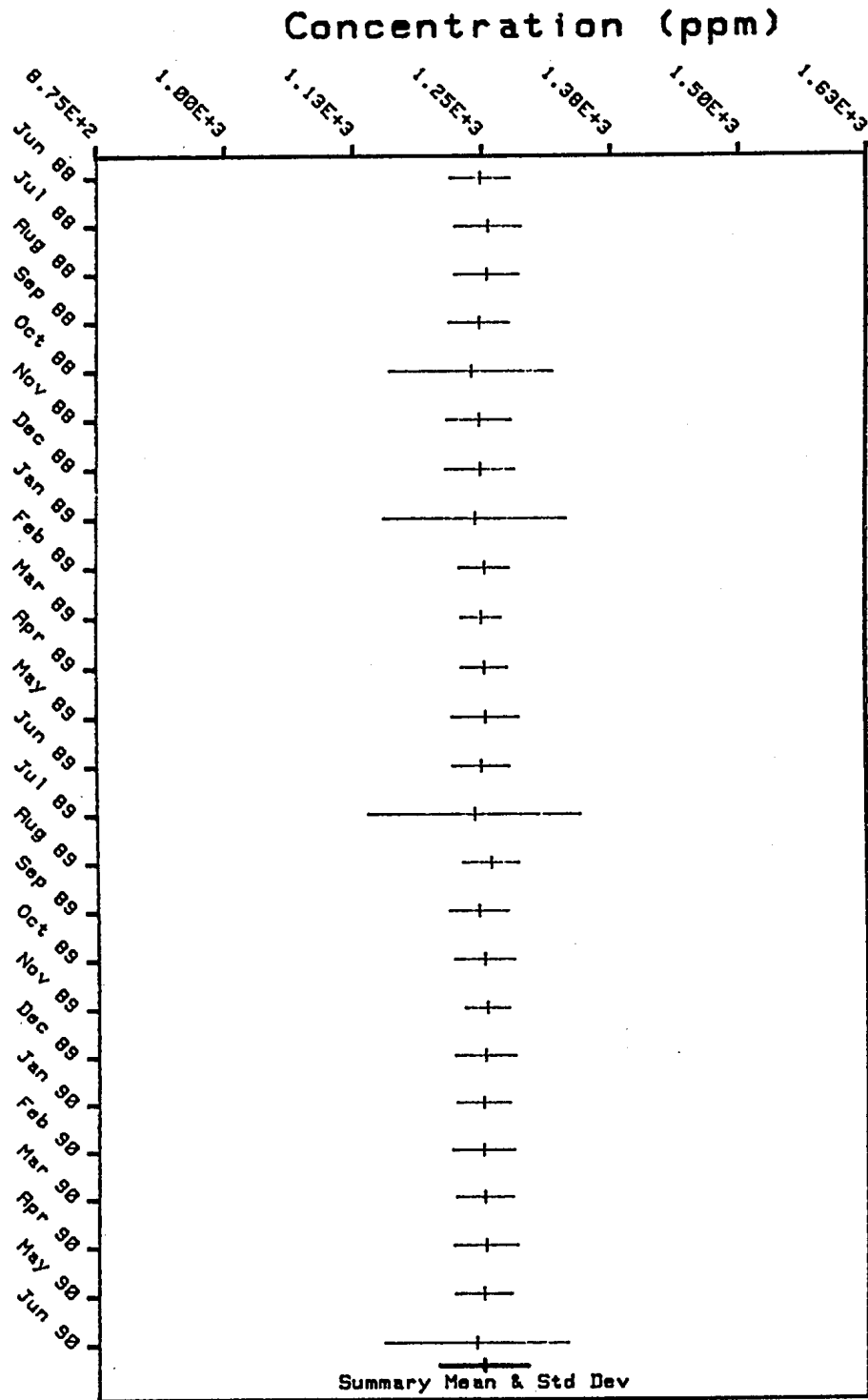


FIGURE II2
Monthly Mean Concentrations and Standard Deviations
in the 1,250 ppm Tetrafluoroethylene Female Rat Exposure Chamber
for the 2-Year Study

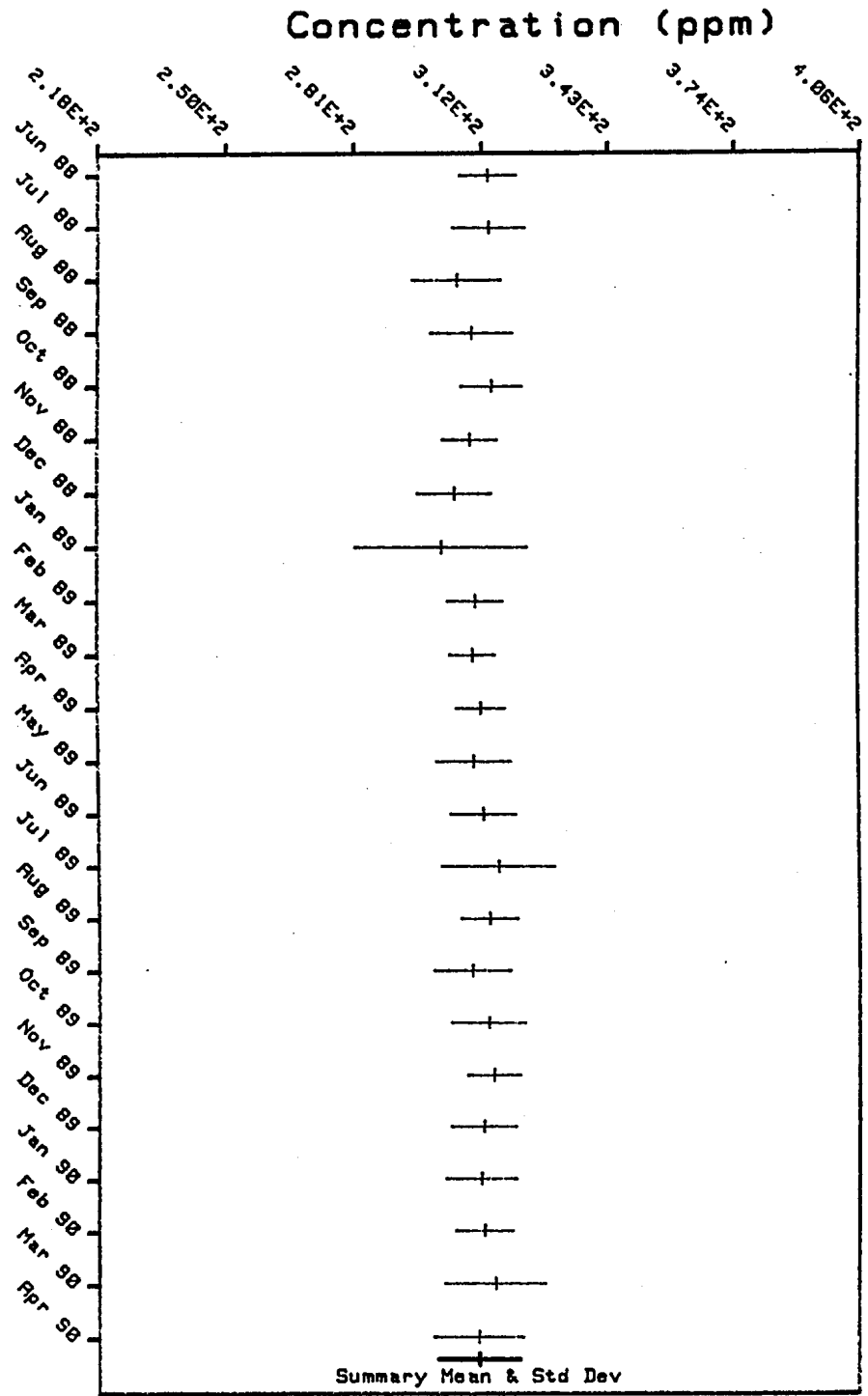


FIGURE I13
 Monthly Mean Concentrations and Standard Deviations
 in the 312 ppm Tetrafluoroethylene Mouse Exposure Chamber
 for the 2-Year Study

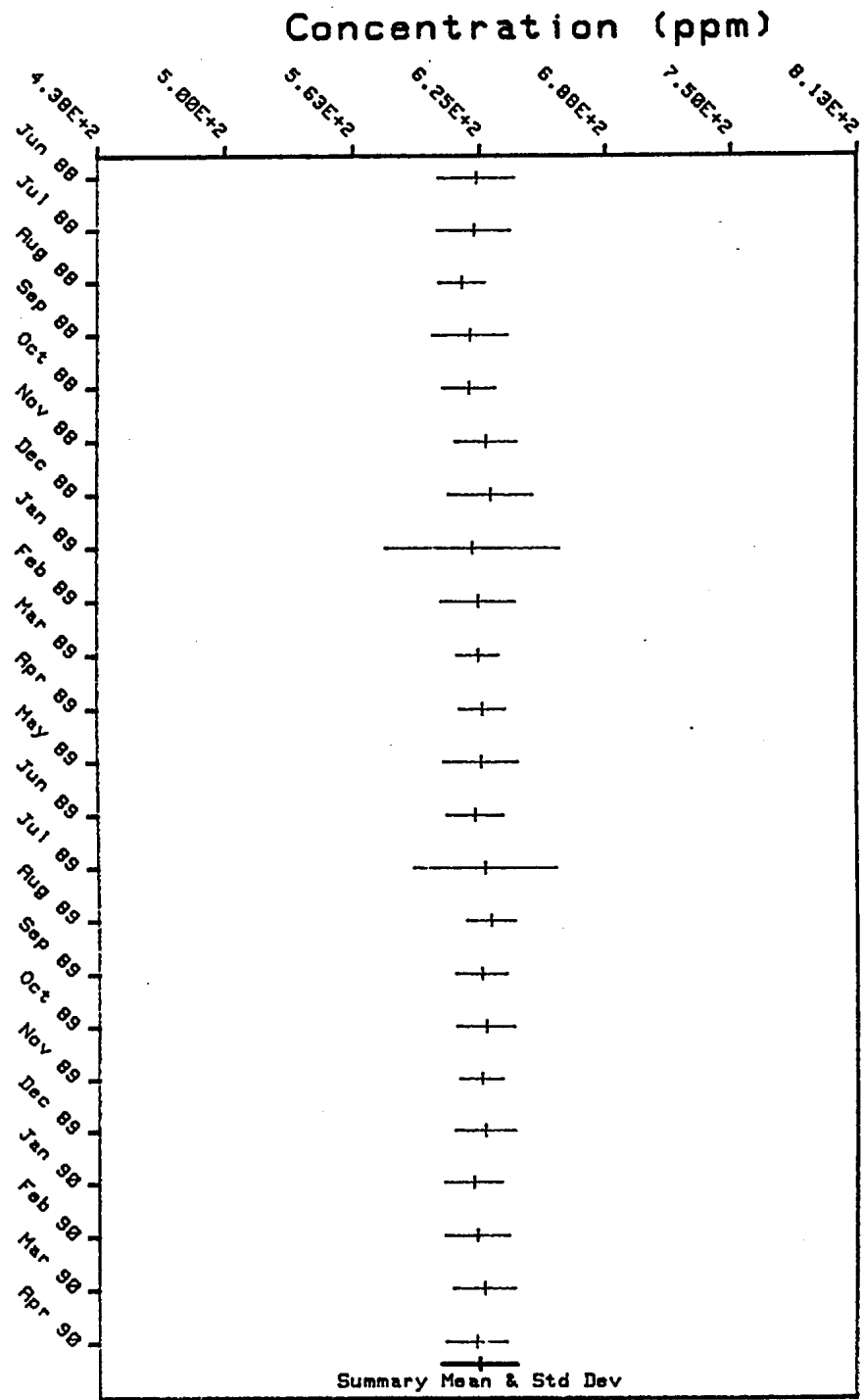


FIGURE I14
Monthly Mean Concentrations and Standard Deviations
in the 625 ppm Tetrafluoroethylene Mouse Exposure Chamber
for the 2-Year Study

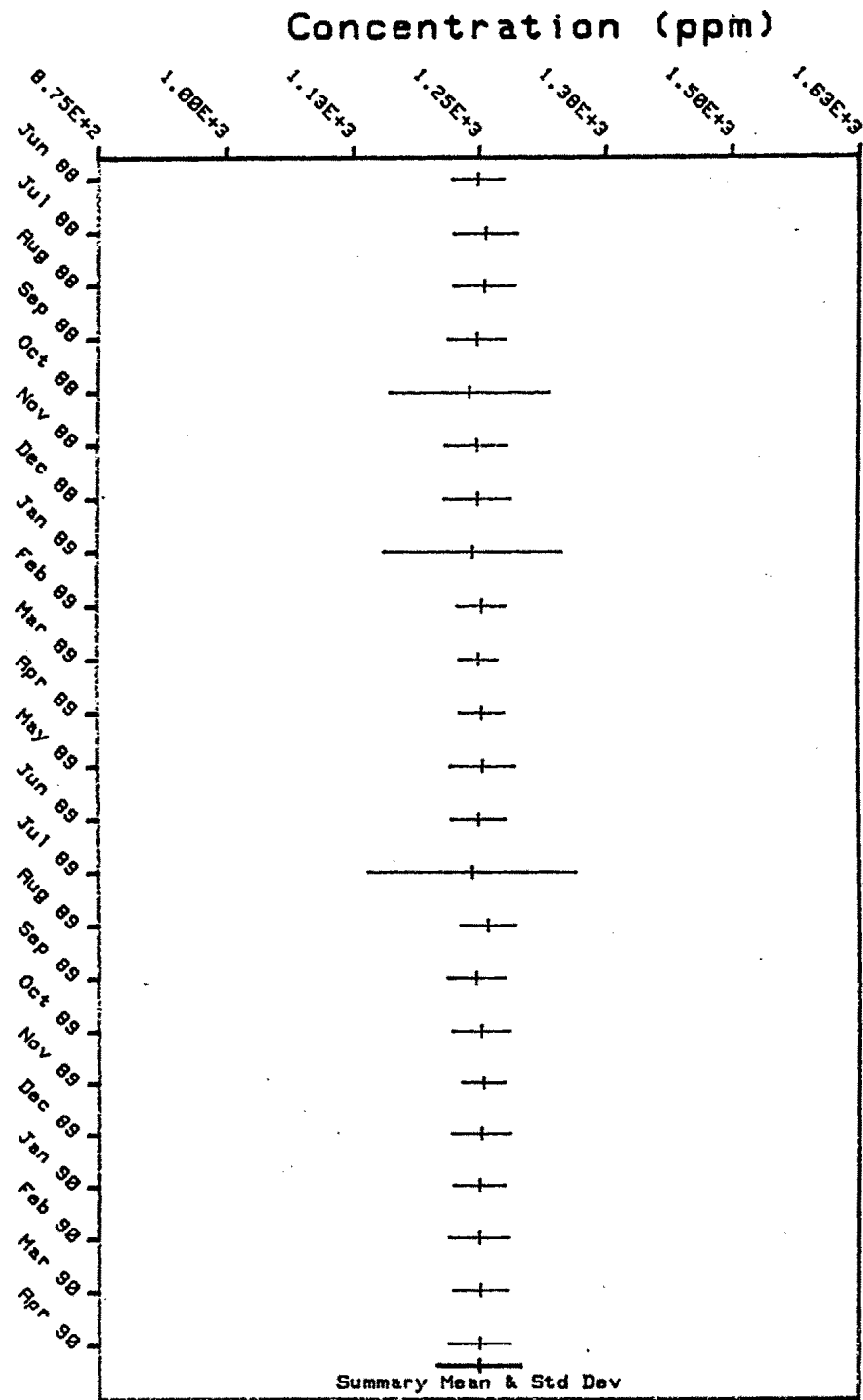


FIGURE I15
 Monthly Mean Concentrations and Standard Deviations
 in the 1,250 ppm Tetrafluoroethylene Mouse Exposure Chamber
 for the 2-Year Study

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION,
AND CONTAMINANT LEVELS
IN NIH-07 RAT AND MOUSE RATION

| | | |
|-----------------|---|------------|
| TABLE J1 | Ingredients of NIH-07 Rat and Mouse Ration | 300 |
| TABLE J2 | Vitamins and Minerals in NIH-07 Rat and Mouse Ration | 300 |
| TABLE J3 | Nutrient Composition of NIH-07 Rat and Mouse Ration | 301 |
| TABLE J4 | Contaminant Levels in NIH-07 Rat and Mouse Ration | 302 |

TABLE J1
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 | |
| 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 J2
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 |
| <i>d</i> - α -Tocopheryl acetate | 20,000 IU |
| Choline | 560.0 g Choline chloride |
| Folic acid | 2.2 g |
| Niacin | 30.0 g |
| <i>d</i> -Pantothenic acid | 18.0 g <i>d</i> -Calcium pantothenate |
| Riboflavin | 3.4 g |
| Thiamine | 10.0 g Thiamine mononitrate |
| B ₁₂ | 4,000 μ g |
| Pyridoxine | 1.7 g Pyridoxine hydrochloride |
| Biotin | 140.0 mg <i>d</i> -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 J3
Nutrient Composition of NIH-07 Rat and Mouse Ration

| Nutrient | Mean \pm Standard Deviation | Range | Number of Samples |
|--|-------------------------------|----------------|-------------------|
| Protein (% by weight) | 22.97 \pm 0.81 | 21.7) 24.2 | 24 |
| Crude fat (% by weight) | 5.37 \pm 0.32 | 4.60) 5.90 | 24 |
| Crude fiber (% by weight) | 3.66 \pm 0.39 | 2.80) 4.20 | 24 |
| Ash (% by weight) | 6.62 \pm 0.31 | 6.11) 7.30 | 24 |
| Amino Acids (% of total diet) | | | |
| Arginine | 1.280 \pm 0.083 | 1.110) 1.390 | 11 |
| Cystine | 0.308 \pm 0.071 | 0.181) 0.400 | 11 |
| Glycine | 1.158 \pm 0.048 | 1.060) 1.220 | 11 |
| Histidine | 0.584 \pm 0.027 | 0.531) 0.630 | 11 |
| Isoleucine | 0.917 \pm 0.033 | 0.867) 0.965 | 11 |
| Leucine | 1.975 \pm 0.051 | 1.850) 2.040 | 11 |
| Lysine | 1.274 \pm 0.049 | 1.200) 1.370 | 11 |
| Methionine | 0.437 \pm 0.109 | 0.306) 0.699 | 11 |
| Phenylalanine | 0.999 \pm 0.120 | 0.665) 1.110 | 11 |
| Threonine | 0.904 \pm 0.058 | 0.824) 0.985 | 11 |
| Tryptophan | 0.218 \pm 0.153 | 0.107) 0.671 | 11 |
| Tyrosine | 0.685 \pm 0.094 | 0.564) 0.794 | 11 |
| Valine | 1.086 \pm 0.055 | 0.962) 1.170 | 11 |
| Essential Fatty Acids (% of total diet) | | | |
| Linoleic | 2.407 \pm 0.227 | 1.830) 2.570 | 10 |
| Linolenic | 0.259 \pm 0.065 | 0.100) 0.320 | 10 |
| Vitamins | | | |
| Vitamin A (IU/kg) | 6,771 \pm 1,915 | 4,180) 12,140 | 24 |
| Vitamin D (IU/kg) | 4,450 \pm 1,382 | 3,000) 6,300 | 4 |
| α -Tocopherol (ppm) | 36.12 \pm 9.15 | 22.5) 48.9 | 10 |
| Thiamine (ppm) | 19.00 \pm 2.36 | 16.0) 28.0 | 24 |
| Riboflavin (ppm) | 7.83 \pm 0.92 | 6.10) 9.00 | 11 |
| Niacin (ppm) | 98.64 \pm 25.51 | 65.0) 150.0 | 10 |
| Pantothenic acid (ppm) | 30.55 \pm 3.52 | 23.0) 34.6 | 11 |
| Pyridoxine (ppm) | 9.11 \pm 2.53 | 5.60) 14.0 | 11 |
| Folic acid (ppm) | 2.46 \pm 0.63 | 1.80) 3.70 | 11 |
| Biotin (ppm) | 0.268 \pm 0.047 | 0.190) 0.354 | 11 |
| Vitamin B ₁₂ (ppb) | 40.5 \pm 19.1 | 10.6) 65.0 | 11 |
| Choline (ppm) | 2,991 \pm 382 | 2,400) 3,430 | 10 |
| Minerals | | | |
| Calcium (%) | 1.25 \pm 0.11 | 1.10) 1.54 | 24 |
| Phosphorus (%) | 0.95 \pm 0.032 | 0.890) 1.00 | 24 |
| Potassium (%) | 0.886 \pm 0.063 | 0.772) 0.971 | 9 |
| Chloride (%) | 0.529 \pm 0.087 | 0.380) 0.635 | 9 |
| Sodium (%) | 0.316 \pm 0.033 | 0.258) 0.371 | 11 |
| Magnesium (%) | 0.166 \pm 0.010 | 0.148) 0.181 | 11 |
| Sulfur (%) | 0.272 \pm 0.059 | 0.208) 0.420 | 10 |
| Iron (ppm) | 350.5 \pm 87.3 | 255.0) 523.0 | 11 |
| Manganese (ppm) | 92.48 \pm 5.14 | 81.7) 99.4 | 11 |
| Zinc (ppm) | 59.33 \pm 10.2 | 46.1) 81.6 | 11 |
| Copper (ppm) | 11.81 \pm 2.50 | 8.09) 15.4 | 11 |
| Iodine (ppm) | 3.54 \pm 1.19 | 1.52) 5.83 | 10 |
| Chromium (ppm) | 1.66 \pm 0.46 | 0.85) 2.09 | 11 |
| Cobalt (ppm) | 0.76 \pm 0.23 | 0.49) 1.15 | 7 |

TABLE J4
Contaminant Levels in NIH-07 Rat and Mouse Ration^a

| | Mean ± Standard Deviation ^b | Range | Number of Samples |
|---|---|-----------------|-------------------|
| Contaminants | | | |
| Arsenic (ppm) | 0.23 ± 0.17 | 0.05) 0.60 | 24 |
| Cadmium (ppm) | 0.09 ± 0.02 | 0.05) 0.10 | 24 |
| Lead (ppm) | 0.24 ± 0.18 | 0.10) 1.00 | 24 |
| Mercury (ppm) | 0.05 ± 0.02 | 0.02) 0.11 | 24 |
| Selenium (ppm) | 0.40 ± 0.20 | 0.16) 1.21 | 24 |
| Aflatoxins (ppb) ^c | <5.0 | | 23 |
| Nitrate nitrogen (ppm) ^d | 16.60 ± 4.13 | 8.60) 24.0 | 24 |
| Nitrite nitrogen (ppm) ^d | 0.20 ± 0.17 | 0.10) 0.60 | 24 |
| BHA (ppm) ^e | 1.54 ± 0.65 | 0.10) 3.00 | 24 |
| BHT (ppm) ^e | 1.30 ± 0.62 | 0.10) 3.00 | 24 |
| Aerobic plate count (CFU/g) | 46,404 ± 24,682 | 6,700) 120,000 | 24 |
| Coliform (MPN/g) | 50 ± 224 | 3) 1,100 | 24 |
| <i>Escherichia coli</i> (MPN/g) | 3 ± 0.2 | 3) 4 | 24 |
| <i>Salmonella</i> (MPN/g) | Negative | | 24 |
| Total nitrosoamines (ppb) ^f | 8.05 ± 3.23 | 3.60) 16.50 | 24 |
| <i>N</i> -Nitrosodimethylamine (ppb) ^f | 5.85 ± 2.71 | 2.60) 13.00 | 24 |
| <i>N</i> -Nitrosopyrrolidine (ppb) ^f | 2.20 ± 1.25 | 0.90) 5.20 | 24 |
| Pesticides (ppm) | | | |
| α-BHC | <0.01 | | 24 |
| β-BHC | <0.02 | | 24 |
| γ-BHC | <0.01 | | 24 |
| δ-BHC | <0.01 | | 24 |
| Heptachlor | <0.01 | | 24 |
| Aldrin | <0.01 | | 24 |
| Heptachlor epoxide | <0.01 | | 24 |
| DDE | <0.01 | | 24 |
| DDD | <0.01 | | 24 |
| DDT | <0.01 | | 24 |
| HCB | <0.01 | | 24 |
| Mirex | <0.01 | | 24 |
| Methoxychlor | <0.05 | | 24 |
| Dieldrin | <0.01 | | 24 |
| Endrin | <0.01 | | 24 |
| Telodrin | <0.01 | | 24 |
| Chlordane | <0.05 | | 24 |
| Toxaphene | <0.10 | | 24 |
| Estimated PCBs | <0.20 | | 24 |
| Ronnel | <0.01 | | 24 |
| Ethion | <0.02 | | 24 |
| Trithion | <0.05 | | 24 |
| Diazinon | <0.10 | | 24 |
| Methyl parathion | <0.02 | | 24 |
| Ethyl parathion | <0.02 | | 24 |
| Malathion | 0.24 ± 0.23 | 0.05) 1.00 | 24 |
| Endosulfan I | <0.01 | | 24 |
| Endosulfan II | <0.01 | | 24 |
| Endosulfan sulfate | <0.03 | | 24 |

^a CFU = colony forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride

^b For values less than the limit of detection, the detection limit is given as the mean.

^c No aflatoxin measurement was recorded for the lot milled on 2 October 1989.

^d Sources of contamination: alfalfa, grains, and fish meal

^e Sources of contamination: soy oil and fish meal

^f All values were corrected for percent recovery.

APPENDIX K

SENTINEL ANIMAL PROGRAM

| | |
|--|------------|
| METHODS | 304 |
| TABLE K1 Murine Virus Antibody Determinations for Rats and Mice in the 13-Week and 2-Year Studies of Tetrafluoroethylene | 306 |

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 randomly selected rats and mice during the 13-week and 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to Microbiological Associates, Inc. (Bethesda, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test

Time of Analysis

RATS

13-Week Study

ELISA

| | |
|---|-------------------|
| <i>Mycoplasma arthritidis</i> | Study termination |
| <i>Mycoplasma pulmonis</i> | Study termination |
| PVM (pneumonia virus of mice) | Study termination |
| RCV/SDA | |
| (rat coronavirus/sialodacryoadenitis 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

| | |
|-----------------------|------------------------------|
| <i>M. arthritidis</i> | 22 and 24 months |
| <i>M. pulmonis</i> | 22 and 24 months |
| PVM | 6, 12, 18, 22, and 24 months |
| RCV/SDA | 6, 12, 18, 22, and 24 months |
| Sendai | 6, 12, 18, 22, and 24 months |

Hemagglutination Inhibition

| | |
|-----|------------------------------|
| H-1 | 6, 12, 18, 22, and 24 months |
| KRV | 6, 12, 18, 22, and 24 months |

MICE**13-Week Study**

Complement Fixation

| | |
|--|-------------------|
| LCM (lymphocytic choriomeningitis virus) | Study termination |
|--|-------------------|

ELISA

| | |
|---------------------------------------|-------------------|
| Ectromelia virus | Study termination |
| GDVII (mouse encephalomyelitis virus) | Study termination |
| Mouse adenoma virus | Study termination |
| MHV (mouse hepatitis virus) | Study termination |
| <i>M. arthritidis</i> | Study termination |
| <i>M. pulmonis</i> | Study termination |
| PVM | Study termination |
| Reovirus 3 | Study termination |
| Sendai | Study termination |

Immunofluorescence Assay

| | |
|--|-------------------|
| EDIM (epizootic diarrhea of infant mice) | Study termination |
|--|-------------------|

Hemagglutination Inhibition

| | |
|----------------------------|-------------------|
| K (papovavirus) | Study termination |
| MVM (minute virus of mice) | Study termination |
| Polyoma virus | Study termination |

2-Year Study

ELISA

| | |
|-----------------------|--------------------------|
| Ectromelia virus | 6, 12, 18, and 22 months |
| EDIM | 22 months |
| GDVII | 6, 12, 18, and 22 months |
| LCM | 18 and 22 months |
| MVM | 6 and 12 months |
| Mouse adenoma virus | 6, 12, 18, and 22 months |
| MHV | 6, 12, 18, and 22 months |
| <i>M. arthritidis</i> | 22 months |
| <i>M. pulmonis</i> | 22 months |
| PVM | 6, 12, 18, and 22 months |
| Reovirus 3 | 6, 12, 18, and 22 months |
| Sendai | 6, 12, 18, and 22 months |

Immunofluorescence Assay

| | |
|---------------------|--------------------------|
| EDIM | 6, 12, 18, and 22 months |
| LCM | 6 and 12 months |
| Reovirus 3 | 12 months |
| MVM | 18 and 22 months |
| Mouse adenoma virus | 22 months |

Hemagglutination Inhibition

| | |
|---------------|--------------------------|
| K | 6, 12, 18, and 22 months |
| MVM | 18 months |
| Polyoma virus | 6, 12, 18, and 22 months |

Results of serology tests are presented in Table K1.

TABLE K1
Murine Virus Antibody Determinations for Rats and Mice in the 13-Week and 2-Year Studies
of Tetrafluoroethylene

| Interval | Incidence of Antibody in Sentinel Animals | Positive Serologic Reaction for |
|------------------------|--|------------------------------------|
| 13-Week Studies | | |
| Rats | | |
| Study termination | 0/10 | None positive |
| Mice | | |
| Study termination | 0/10 | None positive |
| 2-Year Studies | | |
| Rats | | |
| 6 Months | 0/10 | None positive |
| 12 Months | 0/10 | None positive |
| 18 Months | 0/11 | None positive |
| 22 Months | 0/2 | None positive |
| 24 Months | 2/10 | <i>M. arthritidis</i> ^a |
| Mice | | |
| 6 Months | 0/16 | None positive |
| 12 Months | 1/16 | Reovirus 3 ^b |
| 18 Months | 0/13 | None positive |
| 22 Months | 0/10 | None positive |

^a Further evaluation of samples positive for *M. arthritidis* by immunoblot and Western blot procedures indicated that the positive titers may be due to cross reaction with antibodies of nonpathogenic *Mycoplasma* or other agents. Only sporadic samples were positive and there were no clinical findings or histopathologic changes of *M. arthritidis* infection in mice with positive titers. Accordingly, *M. arthritidis*-positive titers were considered to be false positives.

^b Negative for Reovirus 3 by Western blot; false positive

H-RAS CODON 61 MUTATION SPECTRA IN HEPATOCELLULAR NEOPLASMS FROM B6C3F₁ MICE EXPOSED TO TETRAFLUOROETHYLENE FOR 2 YEARS

INTRODUCTION

Liver neoplasms are commonly seen in B6C3F₁ mice in 2-year inhalation studies, occurring with a typical incidence of 40% in control males and 20% in control females. Chemical-induced neoplasms in mice have a high frequency of proto-oncogene activation, particularly by point mutations in codon 61 of H-*ras* genes (Maronpot *et al.*, 1995). The frequency of *ras* activation in these neoplasms is often greater than detected in neoplasms occurring in control animals (Reynolds *et al.*, 1987), and there is evidence for chemical specificity in the pattern of oncogene activation (Wiseman *et al.*, 1986; Maronpot *et al.*, 1995). The specific types of oncogene-activating mutations induced by a chemical carcinogen often agree with what is expected based on the DNA adducts formed by the agent (Wiseman *et al.*, 1986). Even for “nongenotoxic carcinogens,” the patterns of *ras* gene mutations in neoplasms can give clues about the mechanism of tumorigenesis (Devereux *et al.*, 1993; Maronpot *et al.*, 1995).

MATERIALS AND METHODS

Liver neoplasms: Male and female B6C3F₁ mice were exposed to 0, 312, 625, or 1,250 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week for 2 years. At necropsy, liver neoplasms were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned to a thickness of 5 μ m, and stained with hematoxylin and eosin (H&E). Subsequently, six unstained serial sections (10 μ m thick) were prepared from paraffin blocks containing hepatocellular adenomas or carcinomas for isolation of DNA for polymerase chain reaction (PCR)-based assays. In order to isolate adequate amounts of DNA, liver neoplasms greater than 1 mm in diameter were identified for analysis. Based on the size criterion, a total of 66 paraffin-embedded neoplasms were examined for genetic alterations in the H-*ras* gene. These included 53 neoplasms from tetrafluoroethylene-exposed mice and 13 neoplasms from control mice. Also, 13 frozen liver neoplasms were evaluated for H-*ras* and K-*ras* mutations. For the liver neoplasms from which frozen and paraffin-embedded tissue were available, both were examined to confirm the results.

DNA isolation: The DNA isolation procedure is described in Marmur (1961) and in Sills *et al.* (1995). The paraffin-embedded tissue was deparaffinized and rehydrated before digesting with proteinase K (Wright and Manos, 1990). The frozen tumor tissue was digested with 10 mg/mL pronase in 1% sodium dodecyl sulfate in TNE buffer (10 mM TRIS; 150 mM NaCl; and 2 mM EDTA disodium salt, pH 7.5). DNA was extracted with phenol and chloroform and precipitated with ethanol. DNA was quantified by optical density at 260 nm and 200 ng/ μ L was used for amplification.

DNA amplification: DNA was amplified by the polymerase chain reaction (Saiki *et al.*, 1988; Sills *et al.*, 1995); details of the use of nested primers are described in Devereux *et al.* (1991, 1993).

Restriction fragment length polymorphic identification: A “cold” method was employed to identify mutation in the H-*ras* gene at codon 61 from liver neoplasms. This was based on restriction fragment length polymorphism in the DNA containing a CAA to AAA, CTA, or CGA mutation in codon 61 of exon 2 (Sukumuar and Barbacid, 1990). The sense primer used for amplification of exon 2 was 5'-GACATCTTAGACACAGCAGTT-3'. A restriction site for MSEI, XbaI, or TaqI enzyme (New England Biolaboratory, Beverly, MA) is created by the presence of a C to A, A to T, or A to G mutation in the first or second base of codon 61, respectively. By using this technique, we detected codon 61 AAA, CTA, and CGA mutations by MSEI, XbaI, and TaqI digestion, respectively; the normal sequence (CAA) of codon 61 is not cut by these enzymes. The reaction was incubated at 37° C (for MSEI or XbaI) or 60° C (for TaqI) for 2 hours. Fifteen μ L of the mixture with bromophenol blue dye was loaded onto the 6% acrylamide TBE gel (8 \times 8 cm \times 1 mm; 15 wells) (Novex, San Diego, CA). The gel was run at 100 volts for 1 hour on the Novex gel electrophoresis unit. Gels were stained with a 5 μ g/mL solution of ethidium bromide for

20 minutes and then destained in distilled water. Ethidium bromide-stained bands were visualized using a 312 nm ultraviolet viewing box and were photographed.

Single-strand conformational analysis: Single-strand conformation analysis (Orita *et al.*, 1989) was performed with PCR products into which [α -³²P]dATP was incorporated during the inner amplification. For the first exon of *K-ras*, 10% acrylamide gel containing 10% glycerol and 1X TRIS-borate-EDTA buffer was electrophoresed at room temperature with constant power at 8 watts for 16 hours on a Model S2 sequencing gel electrophoresis apparatus (Bethesda Research Labs, Gaithersburg, MD). For the second exon of *H-ras*, 12% acrylamide gel with 5% glycerol in 1X TRIS-borate-EDTA buffer was used at 35 watts in a 4 ° C cold room for 5 hours.

RESULTS

In order to determine if the tetrafluoroethylene-induced neoplasms contained an *H-ras* mutation profile similar to that observed with "spontaneous" neoplasms, sample groups of 29 neoplasms consisting of adenomas and carcinomas from the two highest exposure groups, four neoplasms from the low exposure group, and 17 neoplasms from the chamber controls were evaluated by polymerase chain reaction amplification of *H-ras* exon 2 followed by restriction fragment length polymorphism analysis for the three common codon 61 mutations in the B6C3F₁ mouse (Table L1). Single-stranded conformation polymorphism was used as an alternative screening method for detection of *H-* and *K-ras* mutations in DNA from 19 neoplasms following tetrafluoroethylene exposure and from four chamber controls (Table L2). A low frequency (9/62, 15%) of *H-ras* mutations was detected in hepatocellular neoplasms when compared to the high frequency (10/17 or 59% in controls from the present study and 183/333 or 56% in historical controls) detected in spontaneous liver neoplasms from B6C3F₁ mice (Table L1). In addition, the proportion of *H-ras* mutations in hepatocellular neoplasms was 1:1:1 for codon 61 AAA, CGA, and CTA mutations, respectively, when compared to 3:6:1 for controls from the present study or 5:2:1 for spontaneous hepatocellular neoplasms in historical controls. There were generally no differences in the mutation frequency and spectrum between exposure groups and between benign and malignant hepatocellular neoplasms (Tables L1 and L3). *K-ras* mutations were not detected in hepatocellular neoplasms following tetrafluoroethylene exposure.

DISCUSSION

The low frequency (9/62, 15%) of *H-ras* mutations detected in hepatocellular neoplasms following tetrafluoroethylene exposure is similar to that reported with other nongenotoxic agents such as phenobarbital (7%), chloroform (21%), and ciprofibrate (31%) when compared to the generally higher frequency (183/333, 56%) observed in spontaneous liver neoplasms from B6C3F₁ mice (Fox *et al.*, 1990; Maronpot *et al.*, 1995). Other nongenotoxic hepatocellular carcinogens associated with low frequencies of *H-ras* include hexachlorobenzene (Rumsby *et al.*, 1992), dieldrin, (Bauer-Hofmann *et al.*, 1992), methylclofenapate (Stanley *et al.*, 1994), and oxazepam (NTP, 1993). Nongenotoxic agents, by definition, are not positive in *in vitro* mutation assays, and therefore the parent chemical or metabolites do not react with the DNA directly to cause mutations. Our findings of a decreased frequency of *ras* mutations at codon 61 are consistent with the lack of mutagenicity in the *Salmonella typhimurium* assay and suggest that tetrafluoroethylene may be acting in the liver as a nongenotoxic carcinogen. In addition, the finding of no increases in the frequency of micronucleated erythrocytes in peripheral blood samples supports this theory.

The decreased frequency of *H-ras* mutations in tetrafluoroethylene-induced hepatocellular neoplasms is similar to that detected in B6C3F₁ mice exposed to the structurally similar tetrachloroethylene (24%) (Anna *et al.*, 1994). Unlike the lack of *K-ras* mutations in the tetrafluoroethylene study, a few *K-ras* mutations were detected in the tetrachloroethylene study. Data from the present study support the hypothesis that the liver tumors induced by these two structurally similar hepatocellular carcinogens may be acting via a *ras*-independent pathway. Recently, chlordane-induced hepatocarcinogenesis in B6C3F₁ mice was shown to be independent of *ras* activation (Malarkey *et al.*, 1995). Alternate pathways such as cytotoxicity followed by enhanced cell proliferation may also not be playing a major role in the development of hepatocellular neoplasms with tetrafluoroethylene since at 16 days or 13 weeks, there was no evidence of hepatocellular toxicity. Furthermore, since the mutation spectrum at *H-ras* codon 61 was not similar to that of spontaneous hepatocellular neoplasms, the data also argue against the theory that tetrafluoroethylene-induced tumors may be acting by a simple promotional mechanism involving *ras*-mutated cells. If this was

the case, one would expect that a number of the hepatocellular neoplasms would have had similar mutation profiles to those of the controls, due to the clonal expansion of spontaneously initiated cells with *ras* mutations.

The low frequency of H-*ras* mutations in the liver is consistent with the findings with various nongenotoxic carcinogens; however, the variety of different types of neoplasms in the liver of B6C3F₁ mice supports the hypothesis that a potential *in vivo* genotoxic event(s) may be associated with the carcinogenesis process. The multiplicity of hepatocellular neoplasms and the development of liver hemangiosarcomas, and the significant increase in histiocytic sarcomas in the liver suggest that metabolizing enzymes in the liver may have produced unique tetrafluoroethylene metabolites which may have played a role, perhaps through as yet undetected genotoxic events, in the development of the marked increase in tumors in the liver. Similar to tetrafluoroethylene, 1,3-butadiene (Melnick *et al.*, 1990) and vinyl chloride (Froment *et al.*, 1994) induced hepatic hemangiosarcomas and hepatocellular neoplasms and were both genotoxic carcinogens.

REFERENCES

- Anna, C.H., Maronpot, R.R., Pereira, M.A., Foley, J.F., Malarkey, D.E., and Anderson, M.W. (1994). *ras* Proto-oncogene activation in dichloroacetic acid-, trichloroethylene- and tetrachloroethylene-induced liver tumors in B6C3F₁ mice. *Carcinogenesis* **15**, 2255-2261.
- Bauer-Hofmann, R., Buchmann, A., Mahr, J., Kress, S., and Schwarz, M. (1992). The tumour promoters dieldrin and phenobarbital increase the frequency of c-Ha-*ras* wild-type, but not of c-Ha-*ras* mutated focal liver lesions in male C3H/He mice. *Carcinogenesis* **13**, 477-481.
- Devereux, T.R., Anderson, M.W., and Belinsky, S.A. (1991). Role of *ras* protooncogene activation in the formation of spontaneous and nitrosamine-induced lung tumors in the resistant C3H mouse. *Carcinogenesis* **12**, 299-303.
- Devereux, T.R., Foley, J.F., Maronpot, R.R., Kari, F., and Anderson, M.W. (1993). *ras* Proto-oncogene activation in liver and lung tumors from B6C3F₁ mice exposed chronically to methylene chloride. *Carcinogenesis* **14**, 795-801.
- Fox, T.R., Schumann, A.M., Wantanabe, P.G., Yano, B.L., Maher, V.M., and McCormick, J.J. (1990). Mutational analysis of the H-*ras* oncogene in spontaneous C57BL/6 × C3H/He mouse liver tumors and tumors induced with genotoxic and nongenotoxic hepatocarcinogens. *Cancer Res.* **50**, 4014-4019.
- Froment, O., Boivin, S., Barbin, A., Bancel, B., Trepo, C., and Marion, M.-J. (1994). Mutagenesis of *ras* proto-oncogenes in rat liver tumors induced by vinyl chloride. *Cancer Res.* **54**, 5340-5345.
- Malarkey, D.E., Devereux, T.R., Dinse, G.E., Mann, P.C., and Maronpot, R.R. (1995). Hepatocarcinogenicity of chlordane in B6C3F₁ and B6D2F₁ male mice: Evidence for regression in B6C3F₁ mice and carcinogenesis independent of *ras* proto-oncogene activation. *Carcinogenesis* **16**, 2617-2625.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid micro-organisms. *J. Mol. Biol.* **3**, 208-218.
- Maronpot, R.R., Fox, T., Malarkey, D.E., and Goldsworthy, T.L. (1995). Mutations in the *ras* proto-oncogene: Clues to etiology and molecular pathogenesis of mouse liver tumors. *Toxicology* **101**, 125-156.
- Melnick, R.L., Huff, J., Chou, B.J., and Miller, R.A. (1990). Carcinogenicity of 1,3-butadiene in C57BL/6 × C3H F₁ mice at low exposure concentrations. *Cancer Res.* **50**, 6592-6599.
- National Toxicology Program (NTP) (1993). Toxicology and Carcinogenesis Studies of Oxazepam (CAS No. 604-75-1) in Swiss-Webster and B6C3F₁ Mice (Feed Studies). Technical Report Series No. 443. NIH Publication No. 93-3359. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park.

Orita, M., Suzuki, Y., Sekiya, T., and Hayashi, K. (1989). Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* **5**, 874-879.

Reynolds, S.H., Stowers, S.J., Patterson, R.M., Maronpot, R.R., Aaronson, S.A., and Anderson, M.W. (1987). Activated oncogenes in B6C3F1 mouse liver tumors: Implications for risk assessment. *Science* **237**, 1309-1316.

Rumsby, P.C., Evans, J.G., Phillimore, H.E., Carthew, P., and Smith, A.G. (1992). Search for Ha-*ras* codon 61 mutations in liver tumours caused by hexachlorobenzene and Aroclor 1254 in C57BL/10ScSn mice with iron overload. *Carcinogenesis* **13**, 1917-1920.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., and Erlich, H.A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487-491.

Sills, R.C., Hong, H.L., Greenwell, A., Herbert, R.A., Boorman, G.A., and Devereux, T.R. (1995). Increased frequency of K-*ras* mutations in lung neoplasms from female B6C3F1 mice exposed to ozone for 24 or 30 months. *Carcinogenesis* **16**, 1623-1628.

Stanley, L.A., Blackburn, D.R., Devereux, S., Foley, J., Lord, P.G., Maronpot, R.R., Orton, T.C., and Anderson, M.W. (1994). *Ras* mutations in methylclofenapate-induced B6C3F1 and C57BL/10J mouse liver tumours. *Carcinogenesis* **15**, 1125-1131.

Sukumar, S., and Barbacid, M. (1990). Specific patterns of oncogene activation in transplacentally induced tumors. *Proc. Natl. Acad. Sci. USA* **87**, 718-722.

Wiseman, R.W., Stowers, S.J., Miller, E.C., Anderson, M.W., and Miller, J.A. (1986). Activating mutations of the c-Ha-*ras* protooncogene in chemically induced hepatomas of the male B6C3 F₁ mouse. *Proc. Natl. Acad. Sci. USA* **83**, 5825-5829.

Wright, D.K., and Manos, M.M. (1990). Sample preparation from paraffin-embedded tissues. In *PCR Protocols. A Guide to Methods and Applications* (M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, Eds.), pp. 153-158. Academic Press, San Diego, CA.

TABLE L1
H-ras Mutations at Codon 61 in Spontaneously Occurring and Tetrafluoroethylene-Induced Liver Neoplasms from B6C3F₁ Mice

| Treatment | Activated H-ras (%) | H-ras Codon 61 | | |
|----------------------------------|------------------------|----------------|--------------|--------------|
| | | AAA | CGA | CTA |
| Control, Historical ^a | 183/333 (56%) | 106/177 (60%) | 50/177 (28%) | 21/177 (12%) |
| Control | 10/17 (59%) | 3/10 (30%) | 6/10 (60%) | 1/10 (10%) |
| Tetrafluoroethylene (312 ppm) | 0/3 (0%) | — | — | — |
| Tetrafluoroethylene (625 ppm) | 6/29 (21%) | 2/6 (33%) | 2/6 (33%) | 2/6 (33%) |
| Tetrafluoroethylene (1,250 ppm) | 3/27 (10%) | 1/3 (33%) | 1/3 (33%) | 1/3 (33%) |
| Tetrafluoroethylene (Total) | 9/59 (15%) | 3/9 (33%) | 3/9 (33%) | 3/9 (33%) |

^a Data are presented in Maronpotet *al.* (1995).

TABLE L2
Frequency of K-ras Proto-oncogene Activation in Spontaneously Occurring and Tetrafluoroethylene-Induced Liver Neoplasms from B6C3F₁ Mice

| Treatment | K-ras Mutations (%) |
|----------------------------------|---------------------|
| Control, Historical ^a | 3/167 (1.8%) |
| Control ^b | 0/4 (0%) |
| Tetrafluoroethylene ^b | 0/19 (0%) |

^a Data are presented in Maronpotet *al.* (1995).

^b Determined only for first exon of K-ras at codons 12 and 13.

TABLE L3
H-ras Mutations at Codon 61 Detected in Hepatocellular Adenomas and Carcinomas from B6C3F₁ Mice

| Treatment | Activated H-ras (%) | H-ras Codon 61 | | |
|----------------------------|------------------------|----------------|-----|-----|
| | | AAA | CGA | CTA |
| Controls | | | | |
| Hepatocellular Adenoma | 5/11 (46%) | 1 | 3 | 1 |
| Hepatocellular Carcinoma | 5/6 (83%) | 2 | 3 | 0 |
| Tetrafluoroethylene | | | | |
| Hepatocellular Adenoma | 4/20 (20%) | 1 | 2 | 1 |
| Hepatocellular Carcinoma | 5/39 (13%) | 2 | 1 | 2 |