NATIONAL TOXICOLOGY PROGRAM
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No. 386

TOXICOLOGY AND CARCINOGENESIS STUDIES OF TETRANITROMETHANE

(CAS NO. 509-14-8)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

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 chemical 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. All NTP toxicology and carcinogenesis studies are subjected to a comprehensive audit before being presented for public review. This Technical Report has been reviewed and approved by the NTP Board of Scientific Counselors' Peer Review Panel in public session; the interpretations described herein represent the official scientific position of the NTP.

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

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF TETRANITROMETHANE

(CAS NO. 509-14-8)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

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NATIONAL TOXICOLOGY PROGRAM
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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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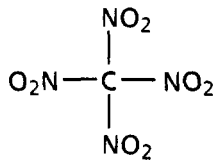

TETRANITROMETHANE

CAS No. 509-14-8

CN_4O_8 Molecular weight 196.0

Synonym: TNM

ABSTRACT

Tetranitromethane is a volatile contaminant formed during the manufacture of TNT and has been used as a rocket fuel and biochemical reagent. Toxicology and carcinogenesis studies were conducted in F344/N rats and B6C3F$_1$ mice of each sex by whole-body exposure to tetranitromethane vapor (greater than 99% pure), 6 hours per day, 5 days per week for 14 days, 13 weeks, or 2 years. Additional groups of male mice were exposed to tetranitromethane for evaluation at 1 year. Genetic toxicology studies were performed in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Fourteen-Day Studies: Exposure concentrations ranged from 2 to 25 ppm for rats and from 2 to 50 ppm for mice. All rats exposed to 25 ppm and all mice exposed at the top concentration of 50 ppm died by day 2; reduced survival was seen in mice exposed to 25 ppm and in rats exposed to 10 ppm. Pulmonary edema in rats and inflammation of the lung in mice were seen in those animals in the 25- and 50-ppm exposure groups examined microscopically.

Thirteen-Week Studies: Exposure concentrations ranged from 0.2 to 10 ppm for rats and mice. No exposure-related deaths occurred in rats. The final mean body weight of rats exposed to 10 ppm was 16% lower than that of controls for males and 6% lower for females. Exposure-related histologic effects included squamous metaplasia of the respiratory epithelium of the nasal mucosa and chronic inflammation of the lung.

No deaths of mice could be clearly related to exposure to tetranitromethane. The final mean body weights of mice exposed to 5 or 10 ppm were 5% or 12% lower than that of controls for males and 9% or 12% lower for females. Exposure-related histologic effects in mice included inflammation and squamous metaplasia of the respiratory epithelium of the nasal mucosa and hyperplasia of the bronchiolar epithelium.

Based on the incidences and severity of lesions in the respiratory tract at the higher concentrations used in the 13-week studies, exposure concentrations chosen for the 2-year studies were 0, 2, and 5 ppm for groups of 50 rats of each sex and 0, 0.5, and 2 ppm for groups of 50 mice of each sex. Additional groups of 6 or 10 male mice were exposed at concentrations of 0, 0.5, or 2 ppm for 1 year.

Body Weights and Survival in the Two-Year Studies: Mean body weights of male and female rats exposed to 5 ppm were approximately 5%-15% lower than those of controls after week 70. Survival of

rats at 104 weeks was as follows: male: control, 18/50; 2 ppm, 17/50; 5 ppm, 4/50; female: 25/50; 34/50; 15/50; survival of rats at the top concentration was reduced due to neoplasia.

Mean body weights of exposed mice were variable and ranged as much as 10% below those of controls during the second year of the studies. Survival of exposed male mice at 104 weeks was significantly lower than that of controls due to neoplasia (control, 37/50; 0.5 ppm, 26/50; 2 ppm, 15/50). Survival of female mice was not significantly affected by exposure to tetranitromethane (31/50; 28/50; 24/50).

Neoplastic and Nonneoplastic Effects in the Two-Year Studies: Effects of exposure to tetranitromethane were limited to the respiratory tract. Hyperplasia of the alveolar and bronchiolar epithelium was observed at increased incidences in exposed rats. The incidence of alveolar/bronchiolar adenomas and carcinomas were markedly increased in exposed male and female rats, with carcinomas (many of which metastasized to other sites) occurring in nearly all rats exposed at the top concentration of 5 ppm (adenomas or carcinomas--male: control, 1/50; 2 ppm, 33/50; 5 ppm, 46/50; female: 0/50; 22/50; 50/50). Many of the rats exposed to 5 ppm also had squamous cell carcinomas of the lung (male: 0/50; 1/50; 19/50; female: 0/50; 1/50; 12/50).

Hyperplasia of the respiratory epithelium and chronic inflammation of the nasal mucosa were observed at increased incidences in exposed male and female rats. Squamous metaplasia of the respiratory epithelium was increased in exposed male rats. No neoplasms of the nasal passage were seen.

In exposed mice, hyperplasia of the alveolar and bronchiolar epithelium was observed at increased incidences. Alveolar/bronchiolar neoplasms, primarily carcinomas (many of which metastasized to other sites), were increased in exposed male and female mice (male: control, 12/50; 0.5 ppm, 27/50; 2 ppm, 47/50; female: 4/49; 24/50; 49/50).

Chronic inflammation of the nasal mucosa and hyperplasia and squamous metaplasia of the respiratory epithelium of the nasal cavity occurred at increased incidences in female mice exposed to 2 ppm. No primary neoplasms of the nasal passage were observed in mice.

Oncogene Analyses: DNA from 14/19 rat and 4/4 mouse lung neoplasms caused morphologic transformation after transfection into cultured NIH/3T3 fibroblasts. The transforming gene from both rat and mouse lung neoplasms was determined by Southern blot analysis to be an activated K-*ras* oncogene. Further studies showed a GC → AT transition in the second base of the 12th codon of the K-*ras* oncogene.

Genetic Toxicology: Tetranitromethane was mutagenic in *S. typhimurium* strains TA98, TA100, and TA1535 with and without exogenous metabolic activation (S9); no mutagenic activity was observed in TA1537 with or without S9. Chromosomal aberrations were observed in CHO cells treated in vitro with tetranitromethane in the presence of S9. Sister chromatid exchanges were induced in CHO cells in the absence of S9.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of tetranitromethane for male and female F344/N rats and male and female B6C3F$_1$ mice, based on increased incidences of alveolar/bronchiolar neoplasms in both species and squamous cell carcinomas of the lung in rats.

Chronic inflammation of the nasal mucosa was related to exposure in rats and female mice, and hyperplasia and squamous metaplasia of the respiratory epithelium were increased in exposed male rats.

SUMMARY OF THE TWO-YEAR INHALATION STUDIES OF TETRANITROMETHANE

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Exposure concentrations			
0, 2, or 5 ppm tetranitro-tetranitro-methane, 6 h/d, 5 d/wk	0, 2, or 5 ppm tetranitro-methane, 6 h/d, 5 d/wk	0, 0.5, or 2 ppm tetranitro-methane, 6 h/d, 5 d/wk	0, 0.5, or 2 ppm methane, 6 h/d, 5 d/wk
Body weights in the 2-year study			
High concentration group lower than controls	High concentration group lower than controls	Exposed groups lower than controls	Exposed groups lower than controls
Survival rates in the 2-year study			
18/50; 17/50; 4/50	25/50; 34/50; 15/50	37/50; 26/50; 15/50	31/50; 28/50; 24/50
Nonneoplastic effects			
Alveolar/bronchiolar hyperplasia; hyperplasia and squamous metaplasia of respiratory epithelium; chronic inflammation of nasal mucosa	Alveolar/bronchiolar hyperplasia; hyperplasia of respiratory epithelium; chronic inflammation of nasal mucosa	Alveolar/bronchiolar hyperplasia	Alveolar/bronchiolar hyperplasia; chronic inflammation of nasal mucosa; squamous metaplasia of respiratory epithelium
Neoplastic effects			
Alveolar/bronchiolar neoplasms (1/50; 33/50; 46/50); lung: squamous cell carcinomas (0/50; 1/50; 19/50), sarcomas (0/50; 0/50; 1/50)	Alveolar/bronchiolar neoplasms (0/50; 22/50; 50/50); lung: squamous cell carcinomas (0/50; 1/50; 12/50), malignant mixed tumors (0/50; 0/50; 1/50), sarcomas (0/50; 0/50; 1/50)	Alveolar/bronchiolar neoplasms (12/50; 27/50; 47/50)	Alveolar/bronchiolar neoplasms (4/49; 24/50; 49/50)
Level of evidence of carcinogenic activity			
Clear evidence	Clear evidence	Clear evidence	Clear evidence

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 9.

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 because of major flaws cannot be evaluated ("Inadequate Study"). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports 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 quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either 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 chemically 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 chemically related.

- **No Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing no chemically 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. This 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:

- The 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 incidences 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);
- The presence or absence of dose relationships;
- The statistical significance of the observed tumor increase;
- The 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.

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of Tetranitromethane is based on 13-week studies that began in May 1981 and ended in August 1981 and on 2-year studies that began in March 1982 and ended in March 1984 at Midwest Research Institute (Kansas City, MO).

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The members of the Peer Review Panel who evaluated the draft Technical Report on tetranitromethane on November 20, 1989, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF TETRANITROMETHANE

On November 20, 1989, the draft Technical Report on the toxicology and carcinogenesis studies of tetranitromethane received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Dr. John Bucher, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (clear evidence of carcinogenic activity for male or female rats, clear evidence of carcinogenic activity for male or female mice).

Dr. Gold, a principal reviewer, agreed with the proposed levels of evidence and suggested noting in the conclusions that markedly increased incidences of carcinomas alone and many metastases were observed. She stated that the allowable worker-exposure level established by the Occupational Safety and Health Administration is close to the tetranitromethane concentrations that induced neoplasms in rodents and that this should be pointed out in the Discussion; Dr. Bucher concurred.

Dr. Ashby, the second principal reviewer, agreed with the conclusions. He said that the carcinogenic response was qualitatively predictable by the chemical structure and mutagenicity data but that the potency of the response was not predictable. He commented on the high levels of alveolar/ bronchiolar neoplasms in control animals, particularly in male mice. Dr. Bucher replied that the control incidences of neoplasms in all sex/species combinations were approximately equal to historical control incidences.

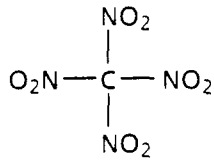
Dr. Zeise, the third principal reviewer, agreed with the conclusions. She noted the possibility that tetranitromethane exposure may have resulted in lung sarcomas in female rats. Dr. Bucher said that the NTP staff was not convinced of an association between chemical exposure and these neoplasms. Dr. Zeise asked whether an epidemiologic study was planned. Dr. Bucher noted that both the Environmental Protection Agency and NIOSH are interested in doing such a study, if an appropriate worker group can be identified.

The discussion centered around the issue of including data on nonneoplastic lesions of the nasal passage in the Abstract and Conclusions. Dr. S. Eustis, NIEHS, commented that incidence rates alone were not very informative without measures of severity for the irritation or injury. All nonneoplastic lesions are graded by the original study pathologist, and when it is considered relevant to the interpretation of effects, information is added in the text.

Dr. Gold moved that the Technical Report on tetranitromethane could be accepted with the conclusions as written for male and female rats and mice, clear evidence of carcinogenic activity, and with mention of inflammation of the nasal mucosa in rats and female mice and nonneoplastic lesions of the respiratory epithelium in rats. Dr. Ashby seconded the motion. Dr. Zeise offered an amendment stating that lung sarcomas and mixed malignant neoplasms in rats would be mentioned under neoplastic effects in the summary table in the Abstract. Dr. Silbergeld seconded the amendment, which was accepted by eight affirmative votes to three negative votes (Drs. Gold, Hayden, and Klaassen). The Panel then unanimously accepted the original motion by Dr. Gold.

I. INTRODUCTION

I. INTRODUCTION



TETRANITROMETHANE

CAS No. 509-14-8

CN_4O_8 Molecular weight 196.0

Synonym: TNM

Physical and Chemical Properties, Use, and Production

Tetranitromethane is a colorless-to-yellow, oily liquid with a pungent, acrid odor. Some physical properties of tetranitromethane are given in Table 1. Tetranitromethane is highly explosive in the presence of impurities and has been used as an oxidizer in rocket propellants, in explosives, and as an additive to increase the cetane number of diesel fuel (Hager, 1949). It has also been used as a chemical reagent for detection of double bonds and as a mild nitrating reagent, reacting with tyrosine residues in proteins (Riordan and Vallee, 1972). Tetranitromethane is also the principal volatile contaminant of TNT (trinitrotoluene) and may constitute as much as 0.12% of the crude material (Moore, 1917).

No current estimates of the amount of tetranitromethane intentionally produced were found in the literature. In Germany during World War II, attempts were made to synthesize large amounts of the chemical for use as a substitute for nitric acid in rocket fuel (Hager, 1949). This method, involving the nitration of acetic anhydride with nitric acid, allowed a production rate of up to 10 tons within a "few weeks" but was costly. By the end of the war, however, a less costly method using acetylene and nitric acid, with a reported capacity of 10 kg/day, was in use.

TABLE 1. SOME PHYSICAL AND CHEMICAL PROPERTIES OF TETRANITROMETHANE (a)

Density at 25° C	1.6229
Boiling point	126° C
Melting point	13.8° C
Viscosity at 20° C	1.76 cp
Soluble	Alcohol, ether
Insoluble	Water

(a) Merck (1983)

Exposure

Current estimates of occupational exposure to tetranitromethane in the United States list 1,445 employees at seven sites as potentially exposed to the chemical (NIOSH, unpublished). Historically, the primary human exposure to tetranitromethane appears to have been during the manufacture and use of TNT (Sievers et al., 1947). TNT is produced by sequential nitration of toluene; the use of strong solutions of nitric acid and high temperatures favors the oxidative destruction of the dinitrotoluene intermediate, leading to formation of tetranitromethane (Sievers et al., 1947; Thompson et al., 1979). During the early part of World War I, there was a high incidence of "TNT intoxication" in U.S. and British plants involved in TNT production; an additional step involving washing the crude material with a sodium sulfite solution to hydrolyze the tetranitromethane was introduced to alleviate this problem. The process used in France also included this washing step (Perkins, 1919).

The signs and symptoms of "TNT intoxication" (caused by inhalation of fumes of crude TNT) included initial nasal irritation, burning of the eyes, dyspnea, cough, tightness in the chest, and dizziness, followed after prolonged exposure by

drowsiness, headache, cyanosis, respiratory distress, and bradycardia (Sievers et al., 1947). Deaths have resulted from severe exposure and were attributed to respiratory failure and methemoglobinemia.

Tetranitromethane has been reported to be an atmospheric pollutant emitted as a byproduct of explosives produced in factories owned by the U.S. government (Thompson et al., 1979). The estimated "worst case" pollutant level of tetranitromethane in the vicinity of the factories was 20 mg/m^3 (about 2.5 ppm). The current time-weighted average/threshold limit value is 1 ppm (8 mg/m^3) (ACGIH, 1988), and the Occupational Safety and Health Administration's permissible exposure limit is also 1 ppm (NIOSH/OSHA, 1981). No quantitative information concerning an odor threshold is available, but the chemical at concentrations in excess of 1 ppm causes lacrimation and upper respiratory irritation and at 0.4 ppm may cause mild irritation (NIOSH/OSHA, 1981).

Absorption, Metabolism, and Short-Term Toxicity

No studies were located in the literature which specifically addressed the absorption, distribution, metabolism, or excretion of tetranitromethane. However, from effects seen after oral administration or inhalation of the chemical, certain information can be inferred. Blood samples obtained 90 minutes after administration of single oral doses of tetranitromethane to Sprague Dawley rats indicated dose-related production of methemoglobin (47% methemoglobin at the LD_{50} dose), suggesting that metabolism could include formation of nitrite ions (Kinkead et al., 1977). After intravenous injection or inhalation exposure, methemoglobin formation was not seen or was reduced when compared with that after oral exposure, suggesting that nitrate reductase activity in the gut may be involved (Kinkead et al., 1977; Vernot et al., 1977).

Early studies of the short-term toxicity of tetranitromethane vapors involved cats, rabbits, and guinea pigs but were only semiquantitative in terms of measurements of tetranitromethane concentrations (Koelsch, 1917). Selected LD_{50} and LC_{50} values are presented in Table 2. At these exposure levels, eye irritation and severe injury to the respiratory tract were consistent findings in all studies involving whole-body inhalation exposure. Lungs appeared congested and had hemorrhagic areas when examined grossly, and they remained distended and exuded a frothy fluid when cut (Horn, 1954; Kinkead et al., 1977). Pulmonary injury was also seen after oral or intravenous exposure. A cat died with gastric hemorrhage and pulmonary edema 5 days after receiving 15 drops of tetranitromethane in alcohol orally (Koelsch, 1917). Tetranitromethane given to rats by intravenous

TABLE 2. SELECTED LD_{50}, LC_{50}, AND ET_{50} VALUES FOR TETRANITROMETHANE (a)

Species	Route of Exposure	Measure
Sprague Dawley male rats	Inhalation	LC_{50} = 17.5 ppm (16.4-18.7) (b)
CF-1 male mice	Inhalation	LC_{50} = 54.4 ppm (48.0-61.7) (b)
Rats (c)	Inhalation	ET_{50} = 1,230 ppm for 36 min (d,e)
Rats (c)	Inhalation	ET_{50} = 300 ppm for 60 min (d,e)
Rats (c)	Inhalation	ET_{50} = 33 ppm for 5.8 h (d,e)
Sprague Dawley male rats	Intravenous	LD_{50} = 12.6 mg/kg (10.0-15.9)
CF-1 male mice	Intravenous	LD_{50} = 63.1 mg/kg (45.0-88.7)
Sprague Dawley male rats	Oral	LD_{50} = 130 mg/kg (83-205)
CF-1 male mice	Oral	LD_{50} = 375 mg/kg (262-511)

(a) Kinkead et al. (1977) unless otherwise specified
(b) Four-hour exposure, 14-day observation
(c) Strain not specified
(d) Time to reach 50% mortality
(e) Horn (1954)

injection caused a foamy nasal discharge and gasping prior to death (Kinkead et al., 1977). Gross observations included pulmonary congestion and hemorrhage. Methemoglobin levels were less than 3%.

Repeated-Exposure Toxicity Studies

In 2-week continuous-exposure inhalation studies conducted with male Sprague Dawley rats exposed to 3.5-7.5 ppm tetranitromethane, lethargy, dyspnea, and increased lung weights were seen at all exposure concentrations (Vernot et al., 1977). Methemoglobin concentrations were not affected. Deaths occurred in rats exposed to 5 ppm and above and appeared directly related to the degree of pulmonary edema present. Evaluation of pulmonary lesions was complicated by chronic murine pneumonia, but catarrhal bronchiolitis, bronchitis, and tracheitis appeared related to chemical exposure. Focal squamous metaplasia was observed in the trachea of rats exposed to 5 or 7.5 ppm.

Horn (1954) exposed 19 rats and 2 dogs to 0 or 6.35 ppm tetranitromethane for 6 hours per day, 5 days per week for 6 months. Eleven rats (vs. 1 control) died during the exposure, but body weight gain of exposed rats was not different from that of controls. Upon gross examination of early-death rats and those killed at the end of the studies, lungs were found to be dark red and distended and exuded edema fluid when cut. Bacterial or viral pneumonia was thought to be the primary cause of early death and was considered to be secondary to the pulmonary irritation caused by tetranitromethane. Both dogs survived. Clinical signs of lethargy and coughing occurred only on the first 2 days of exposure; no gross or microscopic abnormalities were noted in the respiratory tract or in the other organs.

Genetic Toxicity

Little is known about the mutagenic potential of tetranitromethane, except that the short-term test results of the National Toxicology Program show the chemical to be capable of induction of gene mutations in *Salmonella typhimurium* (Zeiger et al., 1987; Table H1) and of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells (Tables H2 and H3). Alper and Ames (1975) reported that tetranitromethane was negative in an assay designed to detect induction of large deletions through the galactose region of the Salmonella chromosome. The urine from workers exposed to TNT in a chemical plant manufacturing munitions was found to be mutagenic to *S. typhimurium* strains TA98 and TA98 NR, strains with and without nitroreductase activity (Ahlborg et al., 1988).

Carcinogenicity

In a pilot epidemiologic study, as reported in a paper concerning the mutagenic activity of metabolites in the urine of workers exposed to TNT, workers exposed to TNT had a higher than expected incidence of stomach cancer (Ahlborg et al., 1988). No human or animal studies of the potential carcinogenicity of tetranitromethane were found in the literature.

Study Rationale

Tetranitromethane was nominated for study by the U.S. Army because of the potential for exposure to workers in the munitions industry and because of the lack of data from long-term toxicity or carcinogenicity studies. Inhalation was chosen as the route of exposure because of the volatility of the chemical and because human exposure would likely occur by this route.

II. MATERIALS AND METHODS

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF TETRANITROMETHANE

Tetranitromethane was obtained in four lots; lot nos. TNM-80-154 and TNM-80-294 were from Hummel Chemical Co., Inc. (South Plainfield, NJ), and lot nos. F101882 and F081882 were from Fluorochem, Inc. (Azusa, CA) (Appendix G). Purity and identity analyses of all lots of the bulk chemical were conducted at Midwest Research Institute (MRI) (Kansas City, MO), except for lot no. TNM-80-154, which was only used in the 14-day studies.

The identity of lots analyzed was confirmed as tetranitromethane by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy, and their purity was determined by titration, thin-layer chromatography, and gas chromatography. The purities of lot nos. TNM-80-294, F101882, and F081882 were determined to be approximately 100%.

Stability studies performed by gas chromatography indicated that tetranitromethane was stable as a bulk chemical when stored protected from light at temperatures up to 25° C. During the toxicology studies, the bulk chemical was stored at 5° C. Periodic analysis by gas chromatography and iodometric titration indicated no notable degradation of the study material throughout the studies.

GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS

Vapor Generation System

Tetranitromethane vapor was generated at room temperature from a gas dispersion bottle by bubbling nitrogen through the liquid (Appendix G). The vapor entered the airstream at the top of the chamber and was mixed and diluted with air in the chamber plenum before entering the chamber (Hazleton 2000®, Lab Products, Inc.) (Table G2). An individual generation system within an isolation box specially designed to operate under negative pressure was used for each exposure chamber.

Vapor Concentration Monitoring

The concentration of tetranitromethane in the study chambers was monitored with a Wilks Miran 1A-CVF Infrared Process Analyzer (14-day studies) or a Miran® II Infrared Gas Analyzer (13-week and 2-year studies) during the 6-hour exposure periods. Samples of each study atmosphere and control atmosphere were analyzed every 10-15 minutes. During the 2-year studies, 94%, 99%, and 98% of the daily mean chamber concentrations for the 0.5-, 2-, and 5-ppm chambers, respectively, were within 10% of the target concentrations. The distribution of the mean daily concentrations in the chambers is summarized in Table G3.

Chamber Atmosphere Characterization

Uniformity of vapor concentration in each exposure chamber was measured periodically throughout the studies. In general, the coefficients of variation of the concentrations determined at the different locations did not exceed 9.4%.

Samples of the 10-ppm tetranitromethane chamber atmosphere were examined for the presence of nitrogen dioxide and nitric acid, the potential degradation products. Colorimetric analysis with calibrated Drager tubes indicated that neither nitric acid nor nitrogen dioxide was present at concentrations greater than 100 ppb or 500 ppb, respectively. Assays for ammonia showed levels of less than 1 ppm with full animal loads in the chambers.

Residual concentrations of tetranitromethane were determined in the chambers after the 6-hour exposure period. The concentrations dropped rapidly; no residual chemical was detected in the chambers after the generators had been stopped and the chambers purged for 1 hour.

FOURTEEN-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories and observed for 4 weeks before being placed on study. The animals were 9-10 weeks old when the studies began.

Groups of five rats of each sex were exposed to air containing tetranitromethane at target concentrations of 0, 2, 5, 10, or 25 ppm, 6 hours per day for 10 days over a 14-day period. Groups of five mice of each sex were exposed to 0, 2, 5, 10, 25, or 50 ppm on the same schedule. Rats and mice were observed once per day and were weighed before exposure, after 1 week, and on day 14. A necropsy was performed on all animals. Histologic examinations were performed on two males and two females in the control groups and one male and one female in the 5-, 10-, and 25-ppm groups of both rats and mice. These exposure groups were chosen for microscopic evaluation because at least one animal from each group had a lesion observed upon gross examination. Animals and tissues examined and details of animal maintenance are presented in Table 3.

THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to tetranitromethane and to determine the concentrations to be used in the 2-year studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories, observed for 20 days, and assigned to groups according to a table of random numbers. Feed was available ad libitum during nonexposure periods; water was available at all times.

Groups of 10 rats and 10 mice of each sex were exposed to air containing tetranitromethane at target concentrations of 0, 0.2, 0.7, 2, 5, or 10 ppm, 6 hours per day, 5 days per week for 13 weeks (65 exposures). Further experimental details are summarized in Table 3.

Animals were observed once per day; moribund animals were killed. Individual animal weights and clinical signs were recorded once per week. At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals. Histologic examinations were performed on all control animals, rats in the 5- and 10-ppm groups, and all exposed mice. Livers were weighed. Tissues and groups examined are listed in Table 3.

ONE-YEAR AND TWO-YEAR STUDIES

Study Design

Groups of 50 rats of each sex were exposed to air containing tetranitromethane at target concentrations of 0 (chamber controls), 2, or 5 ppm, 6 hours per day, 5 days per week for 103 weeks. Groups of 50 mice of each sex were exposed to tetranitromethane at concentrations of 0, 0.5, or 2 ppm on the same schedule. Additional groups of 6 male mice were exposed to 0 or 2 ppm tetranitromethane for 52 weeks, and a group of 10 male mice was exposed to 0.5 ppm tetranitromethane on the same schedule. The number of animals in the 1-year study groups was limited by the chamber capacities. About 6 months into the studies, the 0.5- and 2-ppm mouse and 2-ppm rat inhalation chambers inadvertently received tetranitromethane at these concentrations continuously for 62 hours (October 8-11, 1982). No apparent adverse effects on the animals resulted from this incident. Concentrations measured throughout the studies are summarized in Table G2.

For the 1-year study, 6 male mice from the 0- and 2-ppm groups and 10 male mice from the 0.5-ppm group were selected according to a table of random numbers. Serologic analysis was performed on the six controls and two animals from each dosed group. Histopathologic examinations were performed on all animals.

Source and Specifications of Animals

The male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female $\times$ C3H/HeN MTV$^-$, male) mice used in these studies were produced under strict barrier conditions at Charles River Breeding Laboratories. Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats were shipped to the study laboratory at 4-5 weeks of age and mice at 5-6 weeks of age. Rats were quarantined at the study laboratory for 2 weeks and mice for 3-4 weeks. Thereafter, a complete necropsy was performed on five

TABLE 3. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE INHALATION STUDIES OF TETRANITROMETHANE

Fourteen-Day Studies	Thirteen-Week Studies	One-Year and Two-Year Studies
EXPERIMENTAL DESIGN		
Size of Study Groups		
5 males and 5 females of each species	10 males and 10 females of each species	1 y--6 (control and high dose) or 10 (low dose) male mice; 2 y--50 males and 50 females of each species
Chamber Concentrations		
Rats--0, 2, 5, 10, or 25 ppm tetranitromethane by inhalation; mice--0, 2, 5, 10, 25, or 50 ppm	0, 0.2, 0.7, 2, 5, or 10 ppm tetranitromethane by inhalation	Rats--0, 2, or 5 ppm tetranitromethane by inhalation; mice--0, 0.5, or 2 ppm
Date of First Exposure		
12/3/80	5/19/81	Rats--3/24/82; mice--4/12/82
Date of Last Exposure		
12/16/80	8/18/81	2 y--3/13/84 (rats) or 3/30/84 (mice)
Duration of Exposure		
6 h/d for 10 d over 14 d	6 h/d, 5 d/wk for 13 wk (65 exposures)	6 h/d, 5 d/wk for 52 or 103 wk
Type and Frequency of Observation		
Observed 1 × d; weighed initially and then 1 × wk	Observed 1 × d; weighed initially and 1 × wk thereafter	Observed 2 × d; weighed initially, 1 × wk for 12 wk, and then 1 × mo
Necropsy and Histologic Examinations		
Necropsy performed on all animals; histologic exams performed on 2 males and 2 females from the control groups and 1 male and 1 female from the 5-, 10-, and 25-ppm groups of rats and mice	Necropsy performed on all animals; histologic exams performed on all controls, all rats in the 5- and 10-ppm groups, and all mice. Tissues examined include: adrenal glands, brain, colon, duodenum, esophagus, gallbladder (mice), heart, kidneys, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal passage, pancreas, parathyroid glands, pituitary gland, prostate/testes or ovaries/uterus, rib, salivary glands, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder; livers were weighed at necropsy	2 y--necropsy and histologic exams performed on all animals; the following tissues were examined: adrenal glands, aorta, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/oviduct/uterus, esophagus, femur, heart, ileum, jejunum, kidneys, larynx and pharynx, liver, lungs, mammary gland, mandibular and mesenteric lymph nodes, mesentery, nasal passage, pancreas, parathyroid glands, pituitary gland, preputial or clitoral glands, rectum, rib, salivary glands, sciatic nerve, skeletal muscle, skin, skull, spinal cord, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species		
F344/N rats; $B6C3F_1$ mice	F344/N rats; $B6C3F_1$ mice	1 y--$B6C3F_1$ mice; 2 y--F344/N rats and $B6C3F_1$ mice
Animal Source		
Charles River Breeding Laboratories (Kingston, NY)	Charles River Breeding Laboratories (Kingston, NY)	Charles River Breeding Laboratories (Kingston, NY)
Study Laboratory		
Midwest Research Institute	Midwest Research Institute	Midwest Research Institute
Method of Animal Identification		
Ear tag	Ear tag	Ear tag
Time Held Before Study		
28 d	20 d	Rats--14 d; mice--19 or 26 d

TABLE 3. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE INHALATION STUDIES OF TETRANITROMETHANE (Continued)

Fourteen-Day Studies	Thirteen-Week Studies	One Year and Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Age When Placed on Study		
Rats--9 wk; mice--10 wk	Rats--7-8 wk; mice--8-9 wk	Rats--6-7 wk; mice--8-10 wk
Age When Killed		
Rats--11 wk; mice--12 wk	Rats--20-21 wk; mice--21-22 wk	1 y--60-62 wk; 2 y--110-111 wk (rats) or 112-114 wk (mice)
Necropsy Dates		
12/17/80	8/18/81-8/21/81	1 y--4/12/83; 2 y--3/19/84-3/21/84 (rats) or 4/9/84-4/11/84 (mice)
Method of Animal Distribution		
According to a table of random numbers	Assigned to groups according to a table of random numbers and then placed in cages in numerical order	Same as 13-wk studies
Diet		
NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum except during exposure	Same as 14-d studies	Same as 14-d studies and Rodent Laboratory Chow 5001® meal (Ralston Purina Co., St. Louis, MO) used for a 2-week period; available ad libitum
Bedding		
None	Deotized animal cage board on non-exposure days (Shepherd Specialty Papers, Inc., Kalamazoo, MI)	Same as 13-wk studies
Water		
Tap water in bottles	Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as 13-wk studies
Chambers		
Stainless steel (Young and Berke, Cincinnati, OH)	Same as 14-d studies and stainless steel cage modules (Hazleton 2000®, Lab Products, Inc., City, State)	Rochester-type chambers
Animals per Cage		
5	1	1
Chamber Environment		
Temp--70°-74° F; hum--30%-40%; fluorescent light 12 h/d; approximately 10 chamber air changes/h during exposure	Temp--67°-77.5° F; hum--40%-68%; fluorescent light 12 h/d; 10-15 air changes/h	Temp--59°-81° F; hum--30%-86%; fluorescent light 12 h/d; at least 10 air changes/h

animals of each sex and species to assess their health status. Rats were placed on study at 6-7 weeks of age and mice at 8-10 weeks of age.

Animal Maintenance

Rats and mice were housed individually. Cages were rotated within the inhalation chamber one position clockwise once per week throughout the studies. Serologic analyses were performed as described in Appendix E. Further details of animal maintenance are summarized in Table 3.

Clinical Examinations and Pathology

All animals were observed twice per day. Individual body weights were recorded once per week for the first 12 weeks of the studies and at

least once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed on all animals, including those found dead.

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathologic examinations were performed on all animals (Table 3).

When the pathology evaluation was completed by the laboratory pathologist and the pathology data entered into the Toxicology Data Management System, the slides, paraffin blocks, and residual formalin-fixed tissues were sent to the NTP Archives. The slides, blocks, and residual wet tissues were audited for accuracy of labeling and animal identification and for thoroughness of tissue trimming. The slides, individual animal necropsy records, and pathology tables were sent to an independent pathology quality assessment laboratory. The individual animal records and pathology tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tissues with a tumor diagnosis, all potential target tissues, and all tissues from a randomly selected 10% of the animals were re-evaluated microscopically by a quality assessment pathologist. Nonneoplastic lesions were evaluated for accuracy and consistency of diagnosis only in the potential target organs, in the randomly selected 10% of animals, and in tissues with unusual incidence patterns or trends. Tissues are generally not evaluated in a "blinded" fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle.

The quality assessment report and slides were submitted to a Pathology Working Group (PWG) Chairperson, who reviewed microscopically all potential target tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative examples of potential chemical-related nonneoplastic lesions and neoplasms and examples of disagreements in diagnosis between the laboratory and quality assessment pathologists were shown to the PWG. The PWG included the laboratory pathologist, the quality assessment pathologist, and other pathologists experienced in rodent toxicology, who examined the tissues without knowledge of dose group or previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the diagnosis was changed to reflect the opinion of the PWG. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final pathology data represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Statistical Methods

Survival Analyses: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses if they died from other than natural causes; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. When significant survival differences were detected, additional analyses using these procedures were carried out to determine the time point at which significant differences in the survival curves were first detected. All reported P values for the survival analysis are two-sided.

Calculation of Incidence: The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence: With the exception of lung neoplasms, the majority of tumors in this study were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was a logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor 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 it did not significantly enhance the fit of the model. The dosed 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 tumors are incidental, this comparison of the time-specific tumor prevalences also provides a comparison of the time-specific tumor incidences (McKnight and Crowley, 1984).

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

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

Analysis of Continuous Variables: The statistical analysis of liver weights in the 13-week studies was carried out by using the nonparametric multiple comparison procedures of Dunn (1964) or Shirley (1977) to assess the significance of pairwise comparisons between dosed and control groups. Jonckheere's test (Jonckheere, 1954) was used to evaluate the significance of dose-response trends and to determine whether Dunn's test or Shirley's test was more appropriate for pairwise comparisons.

Historical Control Data: Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984, 1985) are included for those tumors appearing to show compound-related effects.

III. RESULTS

RATS

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs
Survival
Pathology and Statistical Analyses of Results

MICE

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

ONE-YEAR STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs
Survival
Pathology and Statistical Analyses of Results

GENETIC TOXICOLOGY

III. RESULTS: RATS

FOURTEEN-DAY STUDIES

All rats exposed to 25 ppm died within one day (Table 4). Rats exposed to tetranitromethane were lethargic. The final mean body weight of rats exposed to 10 ppm was 34% lower than that of the controls for males and 21% lower for females. The two rats exposed to 25 ppm and examined microscopically had mild-to-moderate pulmonary edema characterized by the accumulation of proteinaceous eosinophilic material in alveoli and in interstitial spaces surrounding bronchioles.

THIRTEEN-WEEK STUDIES

No compound-related deaths occurred (Table 5). The final mean body weight of rats exposed to 10 ppm was 16% lower than that of the controls for males and 6% lower for females. Rats exposed to 10 ppm were lethargic. The absolute and relative liver weights for exposed rats were greater than those for controls (Table 6). No microscopic changes were observed in the liver. Serous exudate was present in the nasal passage in 9/10 male and 8/10 female rats exposed to 10 ppm. Focal squamous metaplasia of the respiratory epithelium of the nasal mucosa was observed in 4/10 female rats exposed to 10 ppm but not in female rats exposed to 5 ppm. The metaplasia generally was mild to moderate in severity and was characterized by replacement of ciliated columnar epithelium by three to five layers of squamous cells. Minimal-to-moderate chronic inflammation of the lung was observed in 10/10 males and 7/10 females exposed to 10 ppm. The lesion consisted of a minimal-to-moderate infiltrate of mononuclear inflammatory cells and minimal fibrosis in the interstitium around the terminal bronchioles.

TABLE 4. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FOURTEEN-DAY INHALATION STUDIES OF TETRANITROMETHANE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)
MALE					
0	5/5	200	228		
2	5/5	199	231		
5	5/5	202	225		
10	(d) 4/5	191	150		
25	(e) 0/5	195	(f)		
FEMALE					
0	5/5	139	154		
2	5/5	135	148		
5	5/5	135	149		
10	5/5	134	121		
25	(e) 0/5	129	(f)		

(a) Number surviving/number initially in group
(b) Initial group mean body weight
(c) Mean body weight change of the group
(d) Day of death: 8
(e) Day of death: all 1
(f) No data are reported due to 100% mortality in this group.

TABLE 5. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE THIRTEEN-WEEK INHALATION STUDIES OF TETRANITROMETHANE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)
MALE					
0	10/10	166 ± 3	376 ± 7	+210 ± 5	
0.2	10/10	164 ± 2	372 ± 4	+208 ± 4	99
0.7	10/10	167 ± 3	361 ± 5	+194 ± 3	96
2	10/10	167 ± 3	373 ± 10	+206 ± 7	99
5	10/10	167 ± 4	367 ± 5	+200 ± 2	98
10	10/10	159 ± 3	316 ± 7	+157 ± 8	84
FEMALE					
0	10/10	118 ± 3	208 ± 5	+90 ± 4	
0.2	10/10	123 ± 3	218 ± 3	+95 ± 3	105
0.7	10/10	123 ± 3	210 ± 4	+87 ± 4	101
2	10/10	123 ± 2	212 ± 5	+89 ± 4	102
5	10/10	119 ± 3	201 ± 4	+82 ± 3	97
10	(d) 9/10	121 ± 3	196 ± 3	+72 ± 3	94

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Accidental death; one animal with an initial weight recorded as 215 g was omitted from the mean for initial weight and weight change.

TABLE 6. LIVER WEIGHTS OF RATS IN THE THIRTEEN-WEEK INHALATION STUDIES OF TETRANITROMETHANE (a)

Concentration (ppm)	Number Weighed	Final Body Weight (grams)	Liver Weight (mg)	Liver Weight/ Final Body Weight (mg/g)
MALE				
0	10	376 ± 6.9	13,470 ± 350	35.9 ± 0.77
0.2	10	372 ± 4.1	**15,500 ± 370	**41.7 ± 0.89
0.7	10	361 ± 4.6	14,220 ± 300	**39.4 ± 0.69
2	10	373 ± 9.5	14,750 ± 350	**39.6 ± 0.46
5	10	367 ± 4.5	*15,560 ± 540	**42.5 ± 1.43
10	10	**316 ± 7.4	13,850 ± 460	**44.2 ± 1.89
FEMALE				
0	10	208 ± 5.0	6,888 ± 229	33.1 ± 0.86
0.2	10	218 ± 2.9	**8,108 ± 211	**37.2 ± 0.70
0.7	10	210 ± 4.1	*8,087 ± 287	**38.5 ± 0.99
2	10	212 ± 4.7	**8,282 ± 232	**39.1 ± 0.80
5	10	201 ± 4.1	7,624 ± 229	**38.0 ± 0.74
10	9	*196 ± 3.3	7,628 ± 201	**38.9 ± 0.52

(a) Mean ± standard error of the mean; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
*P<0.05
**P<0.01

Dose Selection Rationale: Because of lower mean body weight gain and inflammation and fibrosis of the respiratory tract at 10 ppm, the top inhalation exposure concentration selected for rats for the 2-year studies was 5 ppm tetranitromethane, 6 hours per day, 5 days per week. A low exposure concentration of 2 ppm was selected because this was the top concentration for mice, thus permitting exposure of rats and mice in the same chamber.

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of the 5-ppm group of male rats were 7%-17% lower than that of controls after week 84; mean body weights of the 5-ppm group of female rats were 8%-15% lower than that of controls after week 92 (Table 7 and Figure 1). No signs of irritation or other compound-related clinical signs were observed.

TABLE 7. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR INHALATION STUDIES OF TETRANITROMETHANE

Weeks on Study	Chamber Control		2 ppm			5 ppm		
	Av. Wt. (grams)	Number of Survivors	Av. Wt. (grams)	Wt. (percent of chamber controls)	Number of Survivors	Av. Wt. (grams)	Wt. (percent of chamber controls)	Number of Survivors
MALE								
0	141	50	140	99	50	135	96	50
1	167	50	177	106	50	167	100	50
2	198	50	206	104	50	196	99	50
3	224	50	231	103	50	221	99	50
4	246	50	251	102	50	245	100	50
5	266	50	271	102	50	263	99	50
6	282	50	284	101	50	279	99	50
7	297	50	301	101	50	294	99	50
8	312	50	317	102	50	307	98	50
9	319	50	325	102	50	318	100	50
10	332	50	333	100	50	329	99	50
11	338	50	344	102	50	337	100	50
12	346	50	351	101	50	342	99	50
16	368	50	375	102	50	365	99	50
20	387	50	391	101	50	383	99	50
24	406	50	391	96	50	394	97	50
28	425	50	430	101	50	413	97	50
32	425	50	430	101	50	415	98	50
36	438	50	437	100	50	426	97	50
40	444	50	448	101	50	440	99	50
44	446	49	455	102	50	444	100	50
48	454	49	454	100	50	450	99	50
52	458	49	465	102	49	447	98	50
56	464	49	465	100	49	454	98	50
60	466	48	467	100	49	453	97	50
64	478	47	472	99	49	458	96	50
68	481	47	476	99	49	459	95	50
72	484	45	470	97	48	450	93	48
76	481	45	472	98	47	446	93	43
80	479	43	472	99	41	448	94	35
84	474	41	460	97	41	433	91	31
88	468	40	461	99	38	427	91	24
92	456	35	463	102	34	423	93	19
96	459	26	440	96	30	403	88	14
100	447	24	444	99	21	370	83	8
Mean for weeks								
1-12	277		283	102		275	99	
16-52	425		428	101		418	98	
56-100	470		464	99		435	93	
FEMALE								
0	113	50	115	102	50	110	97	50
1	128	49	131	102	50	125	98	50
2	142	49	142	100	50	136	96	50
3	152	49	153	101	50	147	97	50
4	160	49	160	100	50	156	98	50
5	169	49	170	101	50	164	97	50
6	174	49	176	101	50	170	98	50
7	181	49	181	100	50	175	97	50
8	185	49	186	101	50	179	97	50
9	190	49	189	99	50	184	97	50
10	195	49	195	100	50	190	97	50
11	201	49	198	99	50	194	97	50
12	200	49	201	101	50	195	98	50
16	209	49	209	100	50	203	97	50
20	216	49	216	100	50	208	96	50
24	224	49	225	100	50	215	96	50
28	241	49	238	99	50	224	93	50
32	241	49	238	99	50	229	95	50
36	245	48	244	100	50	236	96	50
40	256	48	252	98	50	246	96	50
44	263	48	261	99	50	250	95	50
48	271	48	271	100	50	260	96	50
52	275	48	279	101	50	265	96	50
56	285	48	287	101	50	273	96	50
60	292	48	295	101	50	282	97	50
64	304	48	305	100	49	290	95	50
68	312	47	295	95	49	295	95	49
72	316	46	314	99	49	295	93	48
76	313	43	319	102	49	298	95	45
80	323	41	326	101	47	305	94	43
84	322	41	326	101	43	304	94	39
88	326	37	329	101	41	306	94	37
92	330	34	337	102	39	305	92	35
96	338	28	332	98	39	296	88	30
100	343	26	334	97	37	292	85	22
Mean for weeks								
1-12	173		173	100		168	97	
16-52	244		243	100		234	96	
56-100	317		317	100		295	93	

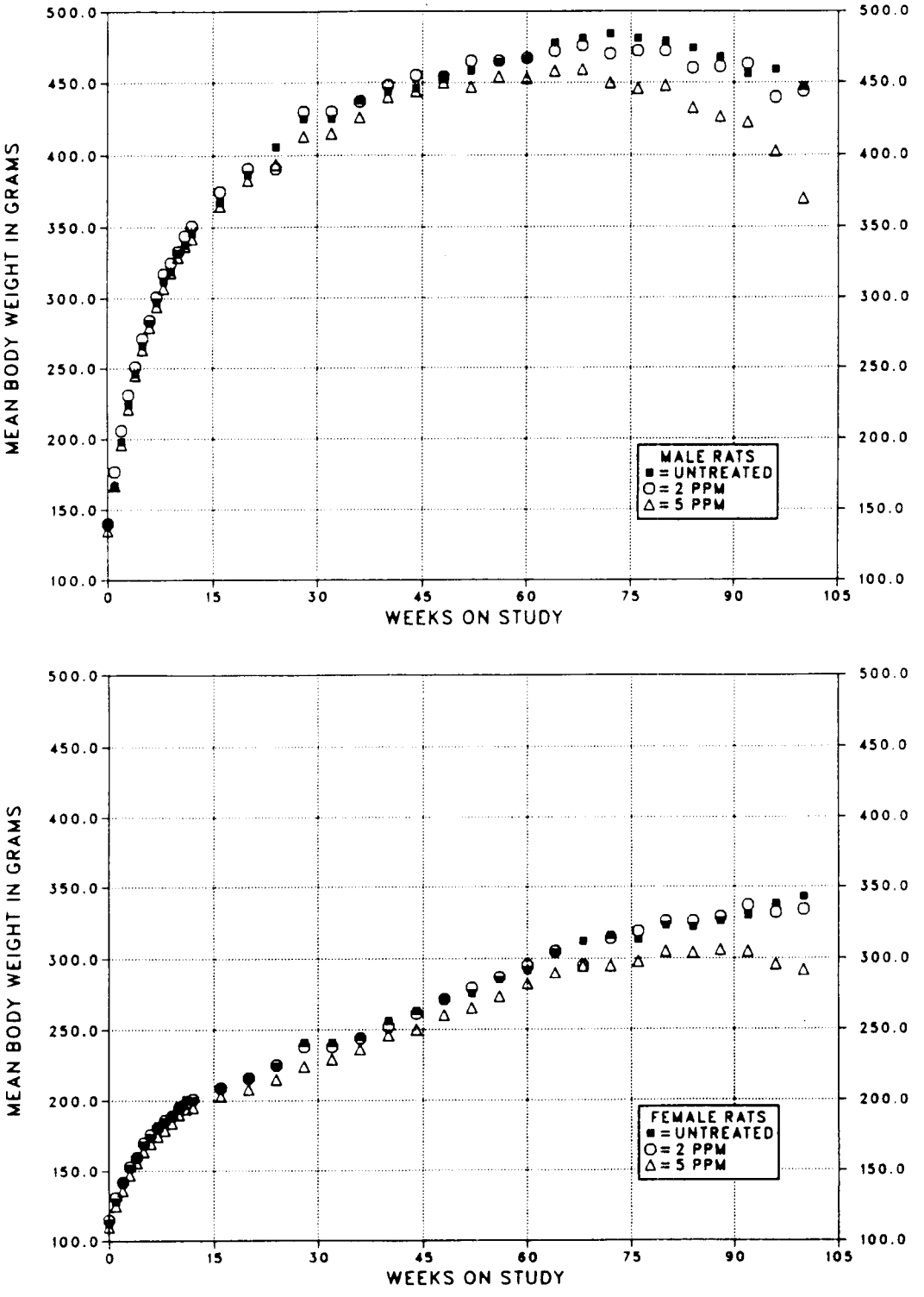


FIGURE 1. GROWTH CURVES FOR RATS EXPOSED TO TETRANITROMETHANE BY INHALATION FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female rats exposed to tetranitromethane at the concentrations used in these studies and for controls are shown in Table 8 and in the Kaplan and Meier curves in Figure 2. Survival of all groups of males was lower than 40%. The survival of the 5-ppm group of males was significantly lower than that of the controls after day 590. No other differences were seen in survival between any groups of either sex.

Pathology and Statistical Analyses of Results

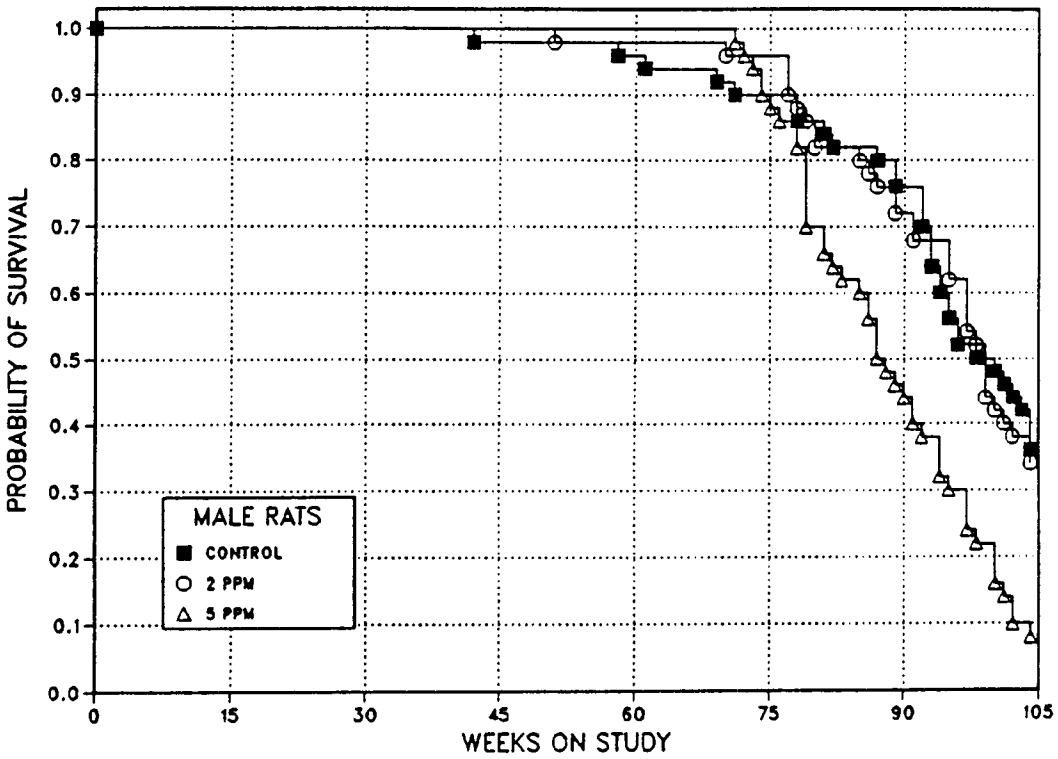
This section describes the statistically significant or biologically noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the lung, nasal passage, adrenal gland, and testis.

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 control incidences for the neoplasms mentioned in this section are presented in Appendixes A and B for male and female rats, respectively.

TABLE 8. SURVIVAL OF RATS IN THE TWO-YEAR INHALATION STUDIES OF TETRANITROMETHANE

	Chamber Control	2 ppm	5 ppm
MALE (a)			
Animals initially in study	50	50	50
Natural deaths	5	8	7
Moribund kills	27	25	39
Animals surviving to study termination	18	17	4
Mean survival (days)	655	660	616
Survival P values (b)	<0.001	0.997	<0.001
FEMALE (a)			
Animals initially in study	50	50	50
Natural deaths	6	3	8
Moribund kills	19	13	27
Animals surviving to study termination	25	34	15
Mean survival (days)	647	691	657
Survival P values (b)	0.063	0.080	0.187

(a) First day of termination period: 727
(b) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.



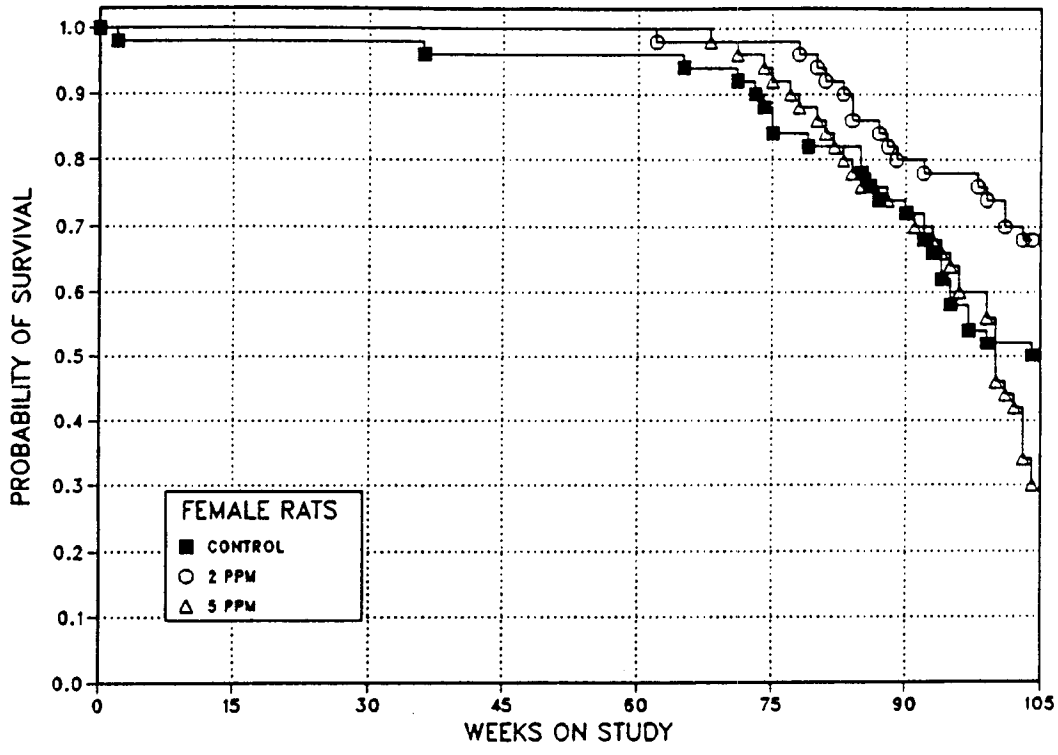


FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES FOR RATS EXPOSED TO TETRANITROMETHANE BY INHALATION FOR TWO YEARS

Lung: Hyperplasia of the alveolar epithelium and bronchiolar epithelium occurred at increased incidences in exposed male and female rats (Table 9). The incidences of alveolar/bronchiolar adenomas or carcinomas (combined) in low dose and high dose males and females and the incidences of squamous cell carcinomas in high dose males and females were significantly greater than those in controls (Table 10). Carcinomas in many exposed rats metastasized; primary metastatic sites were the pancreas, adrenal gland, kidney, heart, and ovary. Hyperplasia, adenoma, and carcinoma are part of a morphologic continuum. Alveolar epithelial hyperplasia was a focal lesion that blended with the surrounding normal lung parenchyma (Figure 3). Normal alveolar architecture was maintained, although alveoli were lined by a single layer of cuboidal cells with basophilic, round or oval nuclei and a moderate amount of eosinophilic cytoplasm. Hyperplasia of the bronchiolar epithelium was characterized by one or more layers of closely packed cuboidal-to-columnar cells that sometimes formed multiple focal clusters or small papillary structures projecting into the airway lumen.

TABLE 9. NUMBERS OF RATS WITH RESPIRATORY TRACT LESIONS IN THE TWO-YEAR INHALATION STUDIES OF TETRANITROMETHANE

Site/Lesion	Male			Female		
	Chamber Control	2 ppm	5 ppm	Chamber Control	2 ppm	5 ppm
Lung						
Number examined	50	50	50	50	50	50
Alveolar epithelium						
Hyperplasia	1	**44	**50	1	**43	**50
Bronchiole						
Hyperplasia	1	**23	**45	0	**28	**48
Alveolar/bronchiolar						
Adenoma						
Single	1	**11	**11	0	*6	3
Multiple	0	2	0	0	0	0
Carcinoma						
Single	0	**18	4	0	**11	3
Two	0	4	**7	0	**7	3
Multiple	0	4	**35	0	1	**44
Metastatic	0	*5	**19	0	0	**15
Squamous cell carcinoma						
Single	0	1	**14	0	1	**10
Two	0	0	*5	0	0	2
Sarcoma	0	0	1	0	0	1
Malignant mixed tumor	0	0	0	0	0	1
Nasal passage						
Number examined	48	49	50	49	50	50
Mucosa						
Chronic inflammation	12	20	**37	13	9	**31
Respiratory epithelium						
Hyperplasia	7	15	**29	5	3	**22
Squamous metaplasia	0	1	**13	0	0	1

*P<0.05 vs. controls
**P<0.01 vs. controls

TABLE 10. LUNG NEOPLASMS IN RATS IN THE TWO-YEAR INHALATION STUDIES OF TETRANITROMETHANE (a)

	Chamber Control	2 ppm	5 ppm
MALE			
Alveolar/Bronchiolar Adenoma			
Overall Rates	1/50 (2%)	13/50 (26%)	11/50 (22%)
Terminal Rates	1/18 (6%)	7/17 (41%)	0/4 (0%)
Day of First Observation	727	535	497
Life Table Tests	P<0.001	P<0.001	P<0.001
Logistic Regression Tests	P=0.015	P<0.001	P=0.005
Alveolar/Bronchiolar Carcinoma			
Overall Rates	0/50 (0%)	26/50 (52%)	46/50 (92%)
Terminal Rates	0/18 (0%)	10/17 (59%)	4/4 (100%)
Day of First Observation		533	497
Life Table Tests	P<0.001	P<0.001	P<0.001
Logistic Regression Tests	P<0.001	P<0.001	P<0.001
Alveolar/Bronchiolar Adenoma or Carcinoma (b)			
Overall Rates	1/50 (2%)	33/50 (66%)	46/50 (92%)
Terminal Rates	1/18 (6%)	11/17 (65%)	4/4 (100%)
Day of First Observation	727	533	497
Life Table Tests	P<0.001	P<0.001	P<0.001
Logistic Regression Tests	P<0.001	P<0.001	P<0.001
Squamous Cell Carcinoma (c)			
Overall Rates	0/50 (0%)	1/50 (2%)	19/50 (38%)
Terminal Rates	0/18 (0%)	1/17 (6%)	1/4 (25%)
Day of First Observation		727	518
Life Table Tests	P<0.001	P=0.489	P<0.001
Logistic Regression Tests	P<0.001	P=0.489	P<0.001
FEMALE			
Alveolar/Bronchiolar Adenoma			
Overall Rates	0/50 (0%)	6/50 (12%)	3/50 (6%)
Terminal Rates	0/25 (0%)	6/34 (18%)	0/15 (0%)
Day of First Observation		727	567
Life Table Tests	P=0.091	P=0.039	P=0.104
Logistic Regression Tests	P=0.208	P=0.039	P=0.116
Alveolar/Bronchiolar Carcinoma			
Overall Rates	0/50 (0%)	19/50 (38%)	50/50 (100%)
Terminal Rates	0/25 (0%)	17/34 (50%)	15/15 (100%)
Day of First Observation		703	356
Life Table Tests	P<0.001	P<0.001	P<0.001
Logistic Regression Tests	P<0.001	P<0.001	P<0.001
Alveolar/Bronchiolar Adenoma or Carcinoma (d)			
Overall Rates	0/50 (0%)	22/50 (44%)	50/50 (100%)
Terminal Rates	0/25 (0%)	20/34 (59%)	15/15 (100%)
Day of First Observation		703	356
Life Table Tests	P<0.001	P<0.001	P<0.001
Logistic Regression Tests	P<0.001	P<0.001	P<0.001
Squamous Cell Carcinoma (e)			
Overall Rates	0/50 (0%)	1/50 (2%)	12/50 (24%)
Terminal Rates	0/25 (0%)	0/34 (0%)	4/15 (27%)
Day of First Observation		639	512
Life Table Tests	P<0.001	P=0.527	P<0.001
Logistic Regression Tests	P<0.001	P=0.478	P<0.001

(a) For a complete explanation of the entries in this table, see Table A3 (footnotes); the statistical analyses used are discussed in Section II (Statistical Methods).
(b) Historical incidence for chamber controls in NTP studies (mean ± SD): 6/347 (2% ± 1%); historical incidence for untreated controls in NTP studies: 44/1,593 (3% ± 2%)
(c) Historical incidence for chamber controls in NTP studies (mean ± SD): 1/347 (0.3% ± 0.8%); historical incidence for untreated controls in NTP studies: 3/1,593 (0.2% ± 0.6%)
(d) Historical incidence for chamber controls in NTP studies (mean ± SD): 4/347 (1% ± 2%); historical incidence for untreated controls in NTP studies: 25/1,639 (2% ± 2%)
(e) Historical incidence for chamber controls: 0/347; historical incidence for untreated controls in NTP studies: 0/1,639

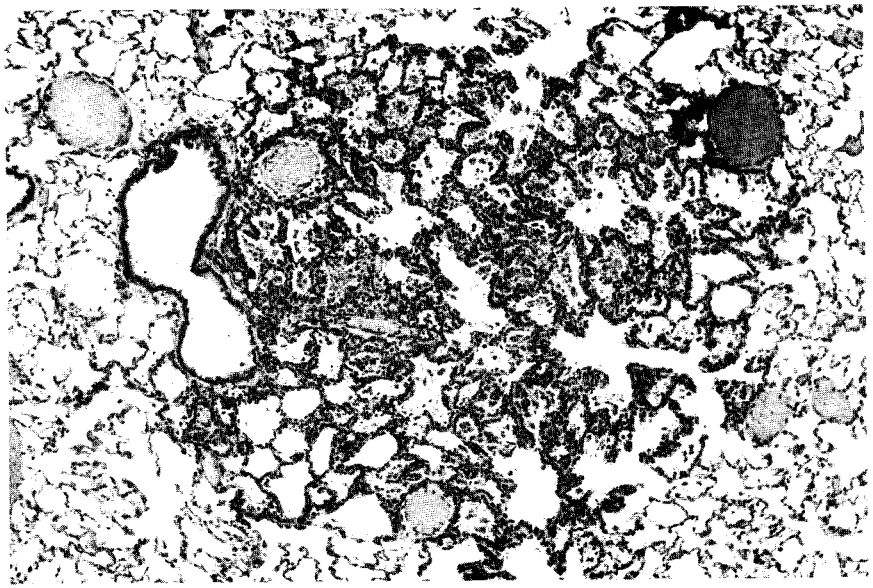


Figure 3. Hyperplasia of the alveolar and bronchiolar epithelium of the lung in a male F344/N rat exposed to 2 ppm tetranitromethane by inhalation for 2 years. The epithelium is thickened and hypercellular, but the normal lung architecture is maintained.

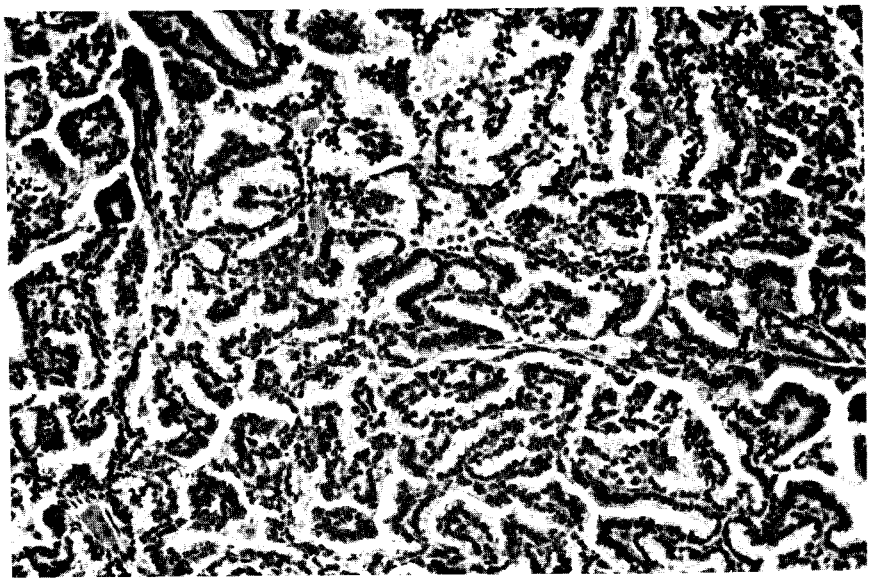


Figure 4. Alveolar/bronchiolar adenoma of the lung in a female F344/N rat exposed to 2 ppm tetranitromethane by inhalation for 2 years. Normal structures are replaced by papillary and tubular structures lined by a single layer of densely packed epithelial cells.

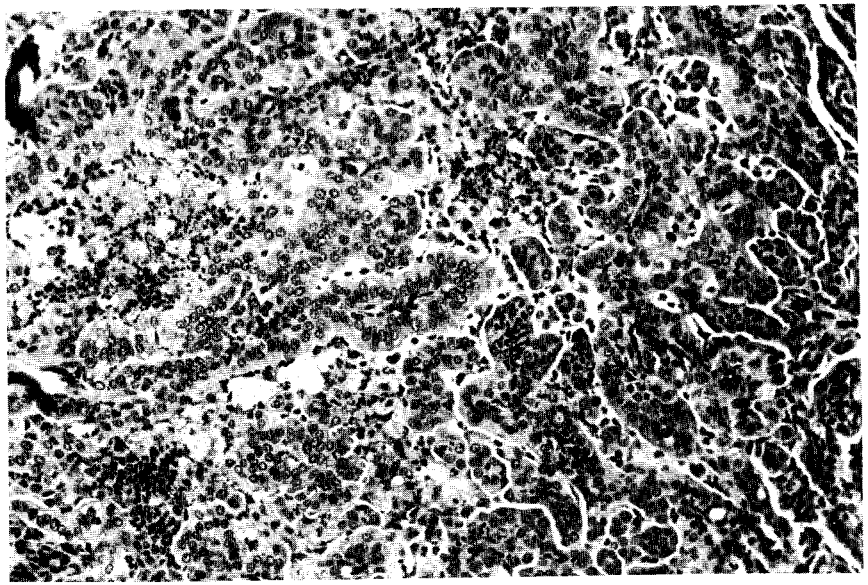


Figure 5. Alveolar/bronchiolar carcinoma of the lung in a female F344/F rat exposed to 5 ppm tetranitromethane by inhalation for 2 years. The neoplastic epithelial cells form solid, haphazardly arranged cords and clusters.

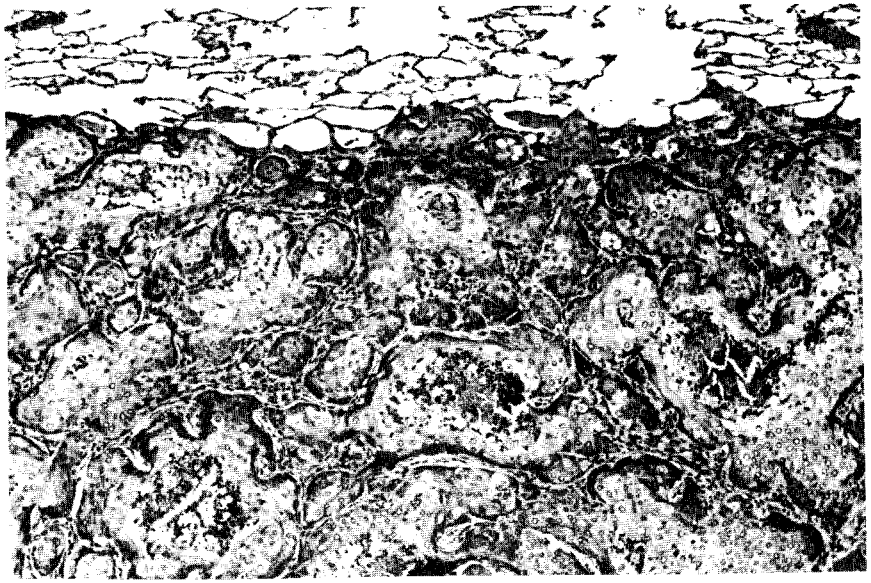


Figure 6. Squamous cell carcinoma of the lung in a male F344/N rat exposed to 5 ppm tetranitromethane by inhalation for 2 years. The neoplasm is composed of numerous irregular clusters of pleomorphic stratified squamous epithelial cells. Some clusters contain a core of keratin and debris.

Alveolar/bronchiolar adenomas were discrete masses that usually compressed the adjacent lung parenchyma (Figure 4). The architecture varied considerably from that of normal lung and consisted of a mixture of complex tubular, papillary, and sometimes alveolar structures that were composed of a core of scant fibrovascular stroma covered by a layer of cuboidal or columnar cells; occasionally, the cells were so densely packed as to assume a multilayered appearance. The neoplastic cells had round or oval nuclei and abundant eosinophilic cytoplasm, sometimes containing one or more clear vacuoles. Mitotic figures were seen infrequently.

Alveolar/bronchiolar carcinomas generally had heterogeneous growth patterns and greater cellular pleomorphism and atypia than adenomas. Carcinomas consisted of tubular, papillary, and alveolar structures and often contained solid sheets, cords, and clusters of highly pleomorphic, polygonal cells with large nuclei and a scant-to-moderate amount of eosinophilic, sometimes vacuolated, cytoplasm (Figure 5). Neoplastic cells in the tubular and alveolar structures generally tended to form multiple layers, and metaplasia of neoplastic cells to stratified squamous epithelium was seen in some carcinomas. Fibrous tissue was sometimes abundant; some carcinomas consisted principally of fibrous tissue. Invasion of pulmonary vessels was sometimes seen, whereas necrosis and inflammation were present in many carcinomas. Squamous cell carcinomas consisted principally of irregular branching cords and clusters of moderately keratinizing stratified squamous epithelium cells (Figure 6). Keratin was abundant in some carcinomas. Since many alveolar/bronchiolar carcinomas contained areas of squamous metaplasia, neoplasms were diagnosed as squamous cell carcinomas only if the majority of the neoplasm was composed of stratified squamous epithelium.

Nasal Passage: Hyperplasia of the respiratory epithelium in low and high dose male and female rats, squamous metaplasia of the respiratory epithelium in high dose males, and inflammation of the nasal mucosa in high dose males and females occurred at increased incidences compared with those in controls. No neoplasms of the nasal passage were seen. Respiratory epithelial hyperplasia generally was mild and consisted of an increase in the number of epithelial cells, producing a slight, irregular thickening of the epithelium. There were increased numbers of goblet cells that occasionally formed intraepithelial glandlike structures. Multiple microcystic spaces filled with lightly eosinophilic material were seen within the hyperplastic epithelium. Some of these microcystic spaces communicated with the nasal lumen. Squamous metaplasia was generally confined to the dorsal and lateral surfaces of the nasal passage, particularly the margin of the naso- and maxilloturbinates, and consisted of replacement of the normal respiratory epithelium by a stratified squamous epithelium that consisted of three to four layers of cells. Inflammation usually was minimal to mild and consisted of varying amounts of exudate within the nasal lumen, sometimes accompanied by an infiltrate of small numbers of neutrophils within the nasal mucosa. Respiratory epithelial hyperplasia and metaplasia and inflammation frequently occurred in the same animal.

Adrenal Gland: Three cortical adenomas and one carcinoma occurred in the 5-ppm group of female rats, but the combined incidence was not significantly greater than in the controls (Table 11). For comparison, the historical incidence of adrenal cortical neoplasms in female F344/N rats in the National Toxicology Program studies is 6/344 (2%) in chamber controls and 53/1,634 (3%) in untreated controls. The highest observed incidence is 2/50 in chamber controls and 6/50 in untreated controls.

Focal hyperplasia and adenomas of the adrenal cortex were observed as a morphologic continuum. Both consisted of small foci of well-differentiated cells that were continuous with the zona fasciculata; contained multiple small, dilated, blood-filled spaces; and extended downward and displaced the cells of the zona reticularis. Foci of hyperplasia generally caused only slight compressions of adjacent tissues and had well-demarcated borders only where they protruded into the zona reticularis. The cords of cells composing these lesions were radially oriented, similar to the normal cords of the zona fasciculata. Adenomas were usually larger than focal hyperplasia, with a well-demarcated border around much of the circumference of the mass. In

TABLE 11. ADRENAL CORTICAL LESIONS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (a)

	Chamber Control	2 ppm	5 ppm
Hyperplasia			
Overall Rates	15/50 (30%)	14/49 (29%)	5/49 (10%)
Adenoma			
Overall Rates	0/50 (0%)	0/49 (0%)	3/49 (6%)
Terminal Rates	0/25 (0%)	0/34 (0%)	1/15 (7%)
Day of First Observation			616
Logistic Regression Tests	P=0.029	(b)	P=0.115
Carcinoma			
Overall Rates	0/50 (0%)	0/49 (0%)	1/49 (2%)
Adenoma or Carcinoma (c)			
Overall Rates	0/50 (0%)	0/49 (0%)	4/49 (8%)
Terminal Rates	0/25 (0%)	0/34 (0%)	1/15 (7%)
Day of First Observation			616
Logistic Regression Tests	P=0.010	(b)	P=0.058

(a) For a complete explanation of the entries in this table, see Table B3 (footnotes); the statistical analyses used are discussed in Section II (Statistical Methods).
(b) No P value is reported because no tumors were observed in the 2-ppm and control groups.
(c) Historical incidence for chamber controls in NTP studies (mean ± SD): 6/344 (2% ± 1%); historical incidence for untreated controls in NTP studies: 53/1,634 (3% ± 3%)

contrast to hyperplasia, the cords of cells composing the mass were more disorganized and generally were not oriented in a radial manner. The carcinoma exhibited moderate cellular pleomorphism and atypia.

Because hyperplasia of the adrenal cortex occurred with a negative trend, and three of the four identified neoplasms met only the minimal criteria for distinguishing an adenoma from hyperplasia, tetranitromethane exposure was not considered to induce proliferative lesions of the adrenal cortex in female rats.

Testis: Although interstitial cell adenomas in male rats occurred with a significant positive trend and the incidence in the 5-ppm group was significantly greater than that in the controls (control, 33/50; 2 ppm, 38/50; 5 ppm, 39/50), these lesions are typically encountered in a high percentage of F344/N rats of comparable age (Table A4); thus, it is unlikely that the small increase in neoplasms observed in this study is an exposure-related effect.

FOURTEEN-DAY STUDIES

All five mice exposed to 50 ppm and 3/5 males and 5/5 females exposed to 25 ppm died before the end of the studies (Table 12). Compound-related clinical signs included lethargy, polypnea, and ataxia. The final mean body weights of males exposed to 5, 10, or 25 ppm were 8%, 11%, or 29% lower than that of controls, and the final mean body weight of females exposed to 10 ppm was 17% lower than that of controls. Reddened lungs were seen in exposed mice at necropsy. Inflammation was observed in the lungs of the three mice exposed to 10 or 25 ppm which lived to the end of the studies and were examined microscopically.

THIRTEEN-WEEK STUDIES

Three mice exposed to tetranitromethane died before the end of the studies (Table 13). The final mean body weights of mice exposed to 5 or 10 ppm were 5% or 12% lower than that of the controls for males and 9% or 12% lower for females. Lethargy and dyspnea in mice exposed to 10 ppm were observed. Relative mean liver weights for all exposed groups of males were greater than those for controls (Table 14). Compound-related histologic effects included inflammation and focal squamous metaplasia (mild) of the respiratory epithelium of the nasal mucosa. Inflammation consisted of minimal-to-mild focal infiltrates of neutrophils in the nasal mucosa; a serous exudate was also present in the nasal passage. Bronchiolar epithelial hyperplasia (mild to moderate) was also exposure related (Table 15). The affected bronchiolar epithelium was thickened, the cells were more columnar, and there was a loss of nuclear polarity.

Dose Selection Rationale: Because of the incidences and severity of inflammation and hyperplastic and metaplastic lesions of the respiratory tract seen at 5 and 10 ppm, the top inhalation

TABLE 12. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FOURTEEN-DAY INHALATION STUDIES OF TETRANITROMETHANE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams)			Final Weight Relative to Controls (percent)
		Initial (b)	Final	Change (c)	
MALE					
0	5/5	26.9	26.7	−0.2	
2	5/5	27.0	26.2	−0.8	98.1
5	5/5	27.2	24.6	−2.6	92.1
10	5/5	26.1	23.8	−2.3	89.1
25	(d) 2/5	27.1	19.0	−8.1	71.2
50	(e) 0/5	26.5	(f)	(f)	(f)
FEMALE					
0	5/5	20.4	21.6	+1.2	
2	5/5	19.5	20.1	+0.6	93.1
5	5/5	19.9	20.4	+0.5	94.4
10	5/5	19.1	17.9	−1.2	82.9
25	(g) 0/5	20.5	(f)	(f)	(f)
50	(e) 0/5	20.4	(f)	(f)	(f)

(a) Number surviving/number initially in group
(b) Initial group mean body weight
(c) Mean body weight change of the group
(d) Day of death: 3,3,7
(e) Day of death: all 2
(f) No data are reported due to 100% mortality in this group.
(g) Day of death: 3,3,3,3,4

TABLE 13. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE THIRTEEN-WEEK INHALATION STUDIES OF TETRANITROMETHANE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)
MALE					
0	10/10	24.7 ± 0.6	32.0 ± 0.6	+7.3 ± 0.4	
0.2	10/10	24.4 ± 0.6	31.2 ± 0.8	+6.8 ± 0.3	98
0.7	(d) 9/10	25.9 ± 0.5	31.5 ± 0.4	+5.8 ± 0.4	98
2	10/10	25.5 ± 0.4	31.1 ± 0.5	+5.6 ± 0.5	97
5	(e) 9/10	25.6 ± 0.5	30.4 ± 0.7	+4.8 ± 0.3	95
10	10/10	25.0 ± 0.5	28.3 ± 0.7	+3.3 ± 0.3	88
FEMALE					
0	10/10	21.3 ± 0.4	28.6 ± 0.7	+7.3 ± 0.6	
0.2	10/10	21.4 ± 0.3	28.9 ± 0.8	+7.5 ± 0.7	101
0.7	10/10	20.6 ± 0.4	27.9 ± 0.4	+7.3 ± 0.5	98
2	10/10	21.7 ± 0.3	28.1 ± 0.5	+6.4 ± 0.5	98
5	10/10	21.5 ± 0.3	26.1 ± 0.5	+4.6 ± 0.3	91
10	(f) 9/10	21.3 ± 0.4	25.1 ± 0.6	+3.6 ± 0.4	88

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Week of death: 3
(e) Week of death: 5
(f) Week of death: 11

TABLE 14. LIVER WEIGHTS OF MICE IN THE THIRTEEN-WEEK INHALATION STUDIES OF TETRANITROMETHANE (a)

Concentration (ppm)	Number Weighed	Final Body Weight (grams)	Liver Weight (mg)	Liver Weight/ Final Body Weight (mg/g)
MALE				
0	10	32.0 ± 0.56	1,540 ± 47	48.2 ± 1.66
0.2	10	31.1 ± 0.76	*1,805 ± 78	**57.8 ± 1.55
0.7	9	31.5 ± 0.38	**1,878 ± 55	**59.7 ± 1.70
2	10	31.1 ± 0.52	**1,933 ± 47	**62.3 ± 1.53
5	9	30.4 ± 0.71	1,686 ± 48	**55.6 ± 1.68
10	10	**28.2 ± 0.68	1,624 ± 40	**57.8 ± 1.93
FEMALE				
0	10	28.5 ± 0.65	1,482 ± 71	51.9 ± 2.01
0.2	10	28.9 ± 0.82	1,706 ± 48	*59.0 ± 1.04
0.7	10	27.9 ± 0.41	1,603 ± 40	57.4 ± 1.21
2	10	28.1 ± 0.55	1,738 ± 56	**61.8 ± 1.05
5	10	**26.1 ± 0.55	1,463 ± 39	56.1 ± 1.19
10	9	**25.1 ± 0.65	1,388 ± 31	55.7 ± 1.73

(a) Mean ± standard error of the mean; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
*P<0.05
**P<0.01

TABLE 15. NUMBERS OF MICE WITH RESPIRATORY TRACT LESIONS IN THE THIRTEEN-WEEK INHALATION STUDIES OF TETRANITROMETHANE (a)

Site/Lesion	Control	0.7 ppm	2 ppm	5 ppm	10 ppm
MALE					
Nasal passage					
Nasal mucosa					
Inflammation	0	0	0	*4	2
Respiratory epithelium					
Squamous metaplasia	0	0	3	**7	3
Lung					
Bronchiolar epithelium					
Hyperplasia	0	0	2	*5	**10
FEMALE					
Nasal passage					
Nasal mucosa					
Inflammation	0	0	0	2	**7
Respiratory epithelium					
Squamous metaplasia	1	0	0	**9	**10
Lung					
Bronchiolar epithelium					
Hyperplasia	0	1	*5	**10	**10

(a) Ten animals were examined in each group.
*P<0.05 vs. controls by Fisher exact test
**P<0.01 vs. controls by Fisher exact test

exposure concentration selected for mice for the 2-year studies was 2 ppm tetranitromethane, 6 hours per day, 5 days per week. A concentration of 0.5 ppm was chosen for the low exposure concentration because the rate of weight gain of male mice in the 13-week study was notably lower than that of controls for groups exposed to 0.7 ppm tetranitromethane or higher.

ONE-YEAR STUDY

Six male controls, 10 males exposed to 0.5 ppm, and 6 males exposed to 2 ppm were evaluated microscopically after 1 year of exposure. Multiple alveolar bronchiolar adenomas were found in the lung of one mouse exposed to 2 ppm. Hepatocellular adenomas were found in the liver of four mice exposed to 0.5 ppm. Hyperplasia of the alveolar epithelium occurred in five mice exposed to 2 ppm, and hyperplasia of the bronchiolar epithelium occurred in two mice in this group. Hyperplasia of the respiratory epithelium was seen in the nasal passage of one mouse exposed to 0.5 ppm.

TWO-YEAR STUDIES

Body Weights and Clinical Signs

During the first year of the studies, the average mean body weights of exposed mice were within 5% of those of controls (Table 16 and Figure 7). During the second year of the studies, the average mean body weights of exposed mice were 5% lower than those of controls for males and 11% or 7% lower for the two groups of exposed female mice. No signs of irritation or other compound-related clinical signs were observed.

TABLE 16. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR INHALATION STUDIES OF TETRANITROMETHANE

Weeks on Study	Chamber Control		0.5 ppm			2 ppm		
	Av. Wt. (grams)	Number of Survivors	Av. Wt. (grams)	Wt. (percent of chamber controls)	Number of Survivors	Av. Wt. (grams)	Wt. (percent of chamber controls)	Number of Survivors
MALE								
0	25.5	56	..	..	..	..	..	..
1	26.3	56	26.2	100	60	26.2	100	56
2	27.8	56	27.3	98	60	27.0	97	56
3	28.7	56	28.5	99	60	27.9	97	56
4	29.2	56	29.1	100	60	28.6	98	56
5	29.7	56	30.2	102	59	29.3	99	56
6	30.0	56	30.2	101	59	29.5	98	56
7	30.4	56	30.7	101	59	30.4	100	56
8	31.0	56	31.0	100	58	30.5	98	56
9	31.1	56	31.3	101	58	31.4	101	56
10	31.7	56	31.4	99	58	31.5	99	56
11	31.3	56	31.2	100	58	32.1	103	56
12	31.6	56	32.3	102	58	31.7	100	56
16-17	32.4	56	32.4	100	58	33.1	102	56
20-21	33.6	56	33.3	99	58	34.3	102	56
24-25	34.8	56	34.2	98	58	35.3	101	55
28-29	35.6	56	34.9	98	58	36.3	102	55
32	37.8	56	35.9	95	58	36.9	98	55
36	38.9	56	35.3	91	58	38.5	99	55
40	40.2	56	37.5	93	58	39.3	98	55
44-45	40.6	56	38.4	95	58	40.6	100	54
48-49	41.0	56	39.6	97	58	40.8	100	54
52-53	41.7	50	39.7	95	48	40.4	97	48
56-57	40.9	50	40.2	98	48	41.4	101	47
60-61	41.2	50	40.8	99	48	42.3	103	47
64-65	41.5	50	39.7	96	47	41.6	100	47
68-69	42.3	50	41.2	97	47	42.3	100	47
72-73	41.4	49	40.5	98	47	41.9	101	47
80-81	42.4	49	39.8	94	46	41.5	98	39
84	42.2	49	39.9	95	42	40.3	95	36
88	42.6	49	38.9	91	41	40.1	94	32
92-93	41.7	46	38.6	93	36	37.1	89	28
96-97	41.4	41	39.4	95	33	35.4	86	23
100-101	41.5	37	39.3	95	27	33.6	81	21
Mean for weeks								
1-12	29.9		30.0	100		29.7	99	
16-53	37.7		36.1	96		37.6	100	
56-101	41.7		39.8	95		39.8	95	
FEMALE								
0	20.8	50	..	..	..	..	..	..
1	21.6	50	21.0	97	50	20.8	96	50
2	23.2	50	22.4	97	50	22.3	96	50
3	24.6	50	23.2	94	50	23.3	95	50
4	24.8	50	24.3	98	50	23.8	96	50
5	25.1	50	24.6	98	50	24.6	98	50
6	25.8	50	25.4	98	50	25.5	99	50
7	26.4	50	25.7	97	50	26.4	100	50
8	26.9	50	26.3	98	50	25.9	96	50
9	26.9	50	27.3	101	50	26.6	99	50
10	27.7	50	27.3	99	50	27.3	99	50
11	27.6	50	27.7	100	50	27.7	100	50
12	27.8	50	27.6	99	50	27.4	99	50
16-17	28.5	50	28.8	101	50	29.3	103	50
20-21	29.5	50	28.7	97	49	29.0	98	50
24-25	30.7	50	29.4	96	49	30.1	98	50
28-29	30.7	50	29.8	97	49	30.2	98	50
32	32.7	49	30.1	92	49	31.7	97	50
36	32.5	49	30.7	94	49	32.2	99	50
40	33.3	49	31.1	93	48	32.9	99	49
44-45	34.2	49	31.3	92	48	33.6	98	49
48-49	34.5	49	31.8	92	47	33.4	97	49
52-53	34.7	49	32.8	95	47	33.4	96	49
56-57	34.9	48	32.8	94	46	34.1	98	49
60-61	35.4	48	32.9	93	46	34.8	98	49
64-65	37.2	48	32.8	88	46	34.4	92	47
68-69	37.4	48	33.6	91	46	34.6	93	47
72-73	36.9	47	33.6	90	46	34.5	93	47
80-81	38.7	45	33.1	86	45	34.7	90	43
84	38.3	45	33.2	87	44	35.2	92	42
88	38.0	40	33.2	87	40	35.5	93	41
92-93	37.2	34	32.6	88	40	34.6	93	39
96-97	37.8	32	33.3	88	38	34.4	91	36
100-101	36.7	32	33.3	91	33	33.4	91	27
Mean for weeks								
1-12	25.7		25.2	98		25.1	98	
16-53	32.1		30.5	95		31.6	98	
56-101	37.1		33.1	89		34.6	93	

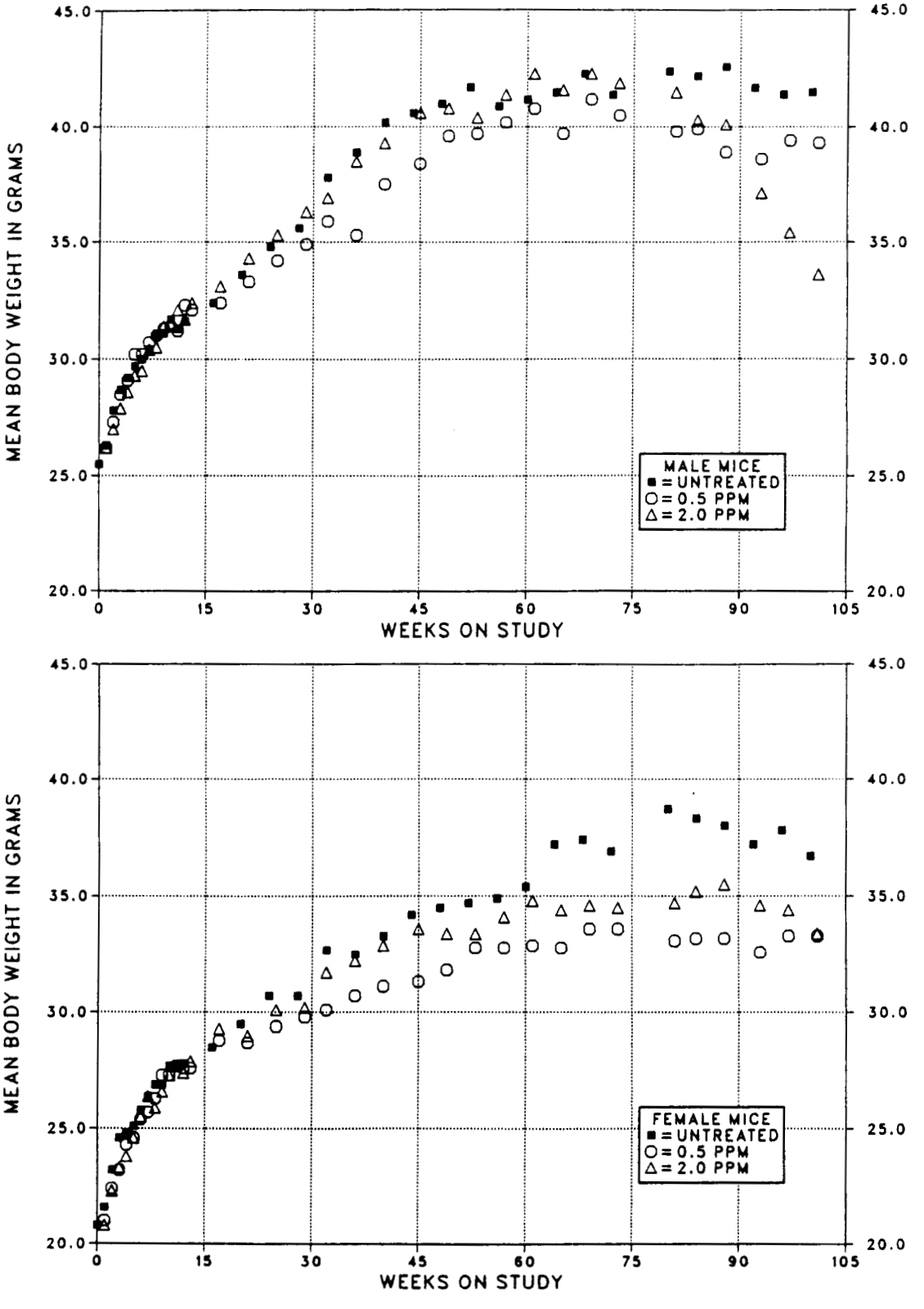


FIGURE 7. GROWTH CURVES FOR MICE EXPOSED TO TETRANITROMETHANE BY INHALATION FOR TWO YEARS

III. RESULTS: MICE

Survival

Estimates of the probabilities of survival for male and female mice exposed to tetranitromethane at the concentrations used in these studies and for controls are shown in Table 17 and in the Kaplan and Meier curves in Figure 8. The survival of the 0.5-ppm group of male mice was significantly lower than that of controls after day 684, and survival of the 2-ppm group of male mice was significantly lower than that of controls after day 546.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the lung and nasal passage.

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 control incidences for the neoplasms mentioned in this section are presented in Appendixes C and D for male and female mice, respectively.

TABLE 17. SURVIVAL OF MICE IN THE TWO-YEAR INHALATION STUDIES OF TETRANITROMETHANE

	Chamber Control	0.5 ppm	2 ppm
MALE (a)			
Animals initially in study	50	50	50
Natural deaths	4	3	19
Moribund kills	9	19	15
Killed accidentally	0	2	1
Animals surviving to study termination	37	26	15
Mean survival (days)	709	650	633
Survival P values (b)	<0.001	0.045	<0.001
FEMALE (a)			
Animals initially in study	50	50	50
Natural deaths	(c) 6	4	10
Moribund kills	12	(c) 18	(c) 17
Killed accidentally	2	1	0
Animals surviving to study termination	31	28	24
Mean survival (days)	672	668	673
Survival P values (b)	0.190	0.703	0.239

(a) First day of termination period: 729
(b) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.
(c) One animal died or was killed in a moribund condition during the termination period and was combined, for statistical purposes, with those killed at termination.

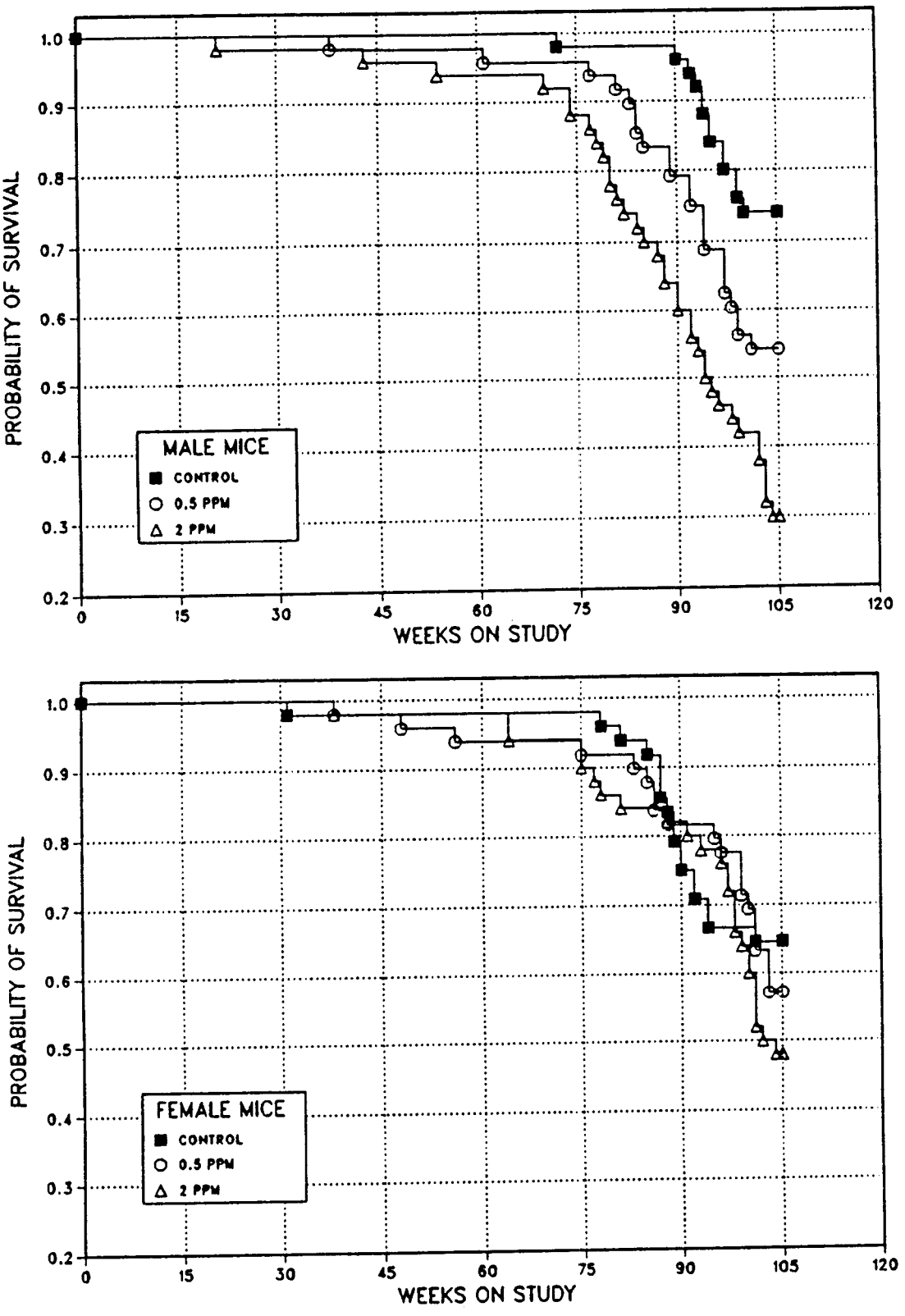


FIGURE 8. KAPLAN-MEIER SURVIVAL CURVES FOR MICE EXPOSED TO TETRANITROMETHANE BY INHALATION FOR TWO YEARS

Lung: Hyperplasia of the alveolar epithelium and bronchioles was observed at increased incidences in exposed mice (Table 18). Histiocytic cellular infiltration of the alveolus was observed at increased incidences in male mice exposed to 2 ppm and in female mice exposed to 0.5 or 2 ppm. Alveolar/bronchiolar adenomas and carcinomas in mice occurred with significant positive trends; the incidences in the exposed groups were significantly greater than those in controls (Table 19). Many of the carcinomas metastasized (Table 18). Most common sites of metastasis were the heart, kidney, and lymph nodes.

Hyperplastic and neoplastic lesions in the lungs of exposed mice generally resembled those in exposed rats.

Nasal Passage: Hyperplasia and squamous metaplasia of the respiratory epithelium occurred at increased incidences in low and high dose female mice (see Table 18). These lesions were similar to but usually less severe than those in rats. An increased incidence of exudate within the nasal lumen was seen in high dose male mice and in low and high dose female mice. The exudate in some affected animals consisted of pale eosinophilic fluid; in most animals, however, the exudate was an admixture of varying numbers of neutrophils and macrophages and debris indicative of the presence of chronic active inflammation of the nasal mucosa. No primary neoplasms of the nasal passage were seen.

TABLE 18. NUMBERS OF MICE WITH RESPIRATORY TRACT LESIONS IN THE TWO-YEAR INHALATION STUDIES OF TETRANITROMETHANE

Site/Lesion	Male			Female		
	Chamber Control	0.5 ppm	2 ppm	Chamber Control	0.5 ppm	2 ppm
Nasal passage						
Number examined	49	50	49	49	50	50
Lumen						
Exudate	1	1	**29	3	**30	**33
Respiratory epithelium						
Hyperplasia	3	6	5	2	5	**17
Squamous metaplasia	0	0	0	0	2	**8
Nasal mucosa						
Chronic inflammation	1	2	5	11	11	*23
Lung						
Number examined	50	50	50	49	50	50
Alveolar epithelium						
Hyperplasia	2	**21	**46	2	**20	**41
Alveolus						
Histiocytic cellular infiltration	7	5	**22	3	*10	**32
Bronchiole						
Hyperplasia	0	**9	**40	0	**7	**41
Alveolar/bronchiolar						
Adenoma						
Single	7	*16	13	1	**12	**10
Multiple	0	1	**21	0	**7	**31
Carcinoma						
Single	6	9	6	3	8	5
Multiple	0	**7	**40	0	3	**40
Metastatic	0	1	**16	0	1	**9

*P<0.05 vs. controls
**P<0.01 vs. controls

TABLE 19. ALVEOLAR/BRONCHIOLAR NEOPLASMS IN MICE IN THE TWO-YEAR INHALATION STUDIES OF TETRANITROMETHANE (a)

	Chamber Control	0.5 ppm (b)	2 ppm (b)
MALE			
Adenoma			
Overall Rates	7/50 (14%)	17/50 (34%)	34/50 (68%)
Terminal Rates	5/37 (14%)	12/26 (46%)	12/15 (80%)
Day of First Observation	662	534	376
Life Table Tests	P<0.001	P=0.002	P<0.001
Logistic Regression Tests	P<0.001	P=0.004	P<0.001
Carcinoma			
Overall Rates	6/50 (12%)	16/50 (32%)	46/50 (92%)
Terminal Rates	5/37 (14%)	8/26 (31%)	15/15 (100%)
Day of First Observation	691	566	485
Life Table Tests	P<0.001	P=0.002	P<0.001
Logistic Regression Tests	P<0.001	P=0.006	P<0.001
Adenoma or Carcinoma (b)			
Overall Rates	12/50 (24%)	27/50 (54%)	47/50 (94%)
Terminal Rates	9/37 (24%)	15/26 (58%)	15/15 (100%)
Day of First Observation	662	534	376
Life Table Tests	P<0.001	P<0.001	P<0.001
Logistic Regression Tests	P<0.001	P<0.001	P<0.001
FEMALE			
Adenoma			
Overall Rates	1/49 (2%)	19/50 (38%)	41/50 (82%)
Terminal Rates	1/31 (3%)	14/28 (50%)	21/24 (88%)
Day of First Observation	729	601	444
Life Table Tests	P<0.001	P<0.001	P<0.001
Logistic Regression Tests	P<0.001	P<0.001	P<0.001
Carcinoma			
Overall Rates	3/49 (6%)	11/50 (22%)	45/50 (90%)
Terminal Rates	2/31 (6%)	8/28 (29%)	23/24 (96%)
Day of First Observation	619	601	444
Life Table Tests	P<0.001	P=0.017	P<0.001
Logistic Regression Tests	P<0.001	P=0.023	P<0.001
Adenoma or Carcinoma (c)			
Overall Rates	4/49 (8%)	24/50 (48%)	49/50 (98%)
Terminal Rates	3/31 (10%)	18/28 (64%)	24/24 (100%)
Day of First Observation	619	601	444
Life Table Tests	P<0.001	P<0.001	P<0.001
Logistic Regression Tests	P<0.001	P<0.001	P<0.001

(a) For a complete explanation of the entries in this table, see Table C3 (footnotes); the statistical analyses used are discussed in Section II (Statistical Methods).
(b) Historical incidence for chamber controls in NTP studies (mean ± SD): 82/398 (21% ± 8%); historical incidence for untreated controls in NTP studies: 277/1,684 (16% ± 7%)
(c) Historical incidence for chamber controls in NTP studies (mean ± SD): 33/396 (8% ± 4%); historical incidence for untreated controls in NTP studies: 107/1,676 (6% ± 4%)

III. RESULTS: GENETIC TOXICOLOGY

Tetranitromethane was tested for mutagenicity in four strains of *Salmonella typhimurium* according to a preincubation protocol with concentrations of 0.03-215 µg/plate in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Zeiger et al., 1987; Table H1). Mutagenic activity was observed in strains TA98, TA100, and TA1535 with and without S9; no increase in mutant colonies occurred in strain TA1537. In cytogenetic tests with Chinese hamster ovary (CHO) cells, tetranitromethane induced sister chromatid exchanges (SCEs) in the absence, but not the presence, of Aroclor 1254-induced male Sprague Dawley rat liver S9 (Table H2). In the second trial without S9, a delayed harvest protocol was used to offset chemical-induced cell cycle delay at the two highest doses, which had also produced a positive response; positive responses occurred at lower doses in the first trial without S9 where normal culture times were used. Chromosomal aberrations were also induced in CHO cells treated with tetranitromethane, but in contrast to the SCE results, positive responses occurred only in the presence of S9 (Table H3); standard harvest times were used for these cultures. The experimental procedures and results are presented in Appendix H.

IV. DISCUSSION AND CONCLUSIONS

IV. DISCUSSION AND CONCLUSIONS

The toxic and carcinogenic properties of tetranitromethane were evaluated by exposing F344/N rats and B6C3F$_1$ mice to vapors of the chemical in 14-day, 13-week, and 2-year studies. Exposure to tetranitromethane at concentrations of 25 ppm or higher caused deaths of male and female rats and mice in 14-day studies. Pulmonary edema in rats and pulmonary inflammation in mice were associated with exposure to the chemical at lethal concentrations. In 13-week studies, no deaths of rats or mice were clearly related to exposure to tetranitromethane at concentrations as high as 10 ppm. Liver weights of exposed rats and mice were somewhat higher than those of controls, but the liver appeared normal microscopically. Changes in the respiratory system were more extensive than those seen in the 14-day studies and appeared related to the exposure concentration in mice. Lesions in rats were found only in the group exposed at the top (10 ppm) concentration; lesions were observed in mice at concentrations as low as 0.7 ppm. Serous exudate was present in the nasal passage of rats and mice, and the nasal mucosa was inflamed in mice. Mild-to-moderate squamous metaplasia of the respiratory epithelium lining the nasal passage was found in rats and mice. In rats, chronic inflammation of the lung was observed and was characterized by infiltration of mononuclear cells and minimal fibrosis of the interstitium in the region of the terminal bronchioles; bronchiolar hyperplasia was seen in mice. Body weights were lower than those in controls only in groups that showed significant pulmonary injury, and there was no evidence of injury to tissues or organs other than to the respiratory system. Exposure to tetranitromethane appeared to have no effect on the skin.

The selection of 5 ppm as the top concentration for rats and 2 ppm for mice in the 2-year studies was based primarily on the belief that the severity of the respiratory tract lesions at higher concentrations in the 13-week studies could prove life threatening if this exposure was continued for 2 years. No effects on body weight or clinical signs of irritation were seen in the short-term studies at the concentrations selected for the 2-year studies.

In the 2-year studies, exposure to 5 ppm resulted in a slightly lower body weight in both male and female rats. This effect became more apparent toward the end of the studies, when survival was declining rather rapidly in these groups. The final survival of rats exposed to 5 ppm was lower than that of controls; in males, this difference was statistically significant. This effect was likely due to the high incidence of lung neoplasms in these animals. The overall survival of male rats was low. A large number of male rats were killed in a moribund condition during the latter part of the study, reflecting an aggressive moribund kill policy in effect at the study laboratory.

Body weights of groups of mice were variable, but weights of exposed mice were generally not more than 5% lower than those of controls until late in the studies. Survival of exposed male mice was lower than that of controls and appeared related to the exposure concentration.

Exposure to tetranitromethane caused a dose-related increase in alveolar/bronchiolar neoplasms to a degree unprecedented in the National Toxicology Program (NTP) studies. Nearly all rats and mice exposed at the top concentrations of 5 and 2 ppm, respectively, including all animals in these groups that survived throughout the 2-year studies, developed alveolar/bronchiolar neoplasms. The incidences of these neoplasms in the low exposure concentration groups (2 ppm for rats and 0.5 ppm for mice) were 66% and 44% in male and female rats and 54% and 48% in male and female mice; these were significant increases over those in the corresponding controls and the historical incidences. The majority of the animals with alveolar/bronchiolar neoplasms had neoplasms diagnosed as carcinomas, and these neoplasms frequently metastasized to a variety of organs. Squamous cell carcinomas of the lung were also markedly increased in rats exposed to 5 ppm (38% in males and 24% in females). This particular type of neoplasm has been found in only 3 of approximately 1,600 untreated control male F344/N rats and in none of a similar number of untreated female controls.

Because the current recommended time-weighted average/threshold limit value for tetranitromethane has been and remains 1 ppm (ACGIH, 1988), the NTP issued an advisory to appropriate Federal agencies on the apparent

carcinogenic hazard of the chemical at this concentration, based on the high frequency of lung nodules in early-death animals (personal communication to R.L. Vance, Occupational Safety and Health Administration, from D.A. Canter, NTP, April 23, 1984). In addition, studies were begun to evaluate pulmonary neoplasms observed in the tetranitromethane studies for the presence of activated oncogenes (Stowers et al., 1987; Appendix I). A brief description of the methods and results follows.

DNA isolated from alveolar/bronchiolar neoplasms in both rats and mice exposed to tetranitromethane and from squamous cell carcinomas in exposed rats was transfected into cultured NIH/3T3 fibroblasts. Morphologic transformation of the fibroblasts was caused by DNA from 14/19 rat neoplasms and 4/4 mouse neoplasms. The transforming gene was identified as a K-*ras* oncogene in both species by Southern blot analysis. The first exon of the K-*ras* gene from normal DNA and that from DNA from two cell lines transformed by tumor DNA were cloned, and the sequences were compared. Both transfectant DNAs had a GC → AT transition in the 2d base of the 12th codon. It has been reported that approximately 40% of examined human pulmonary adenocarcinomas contain an activated Ki-*ras* oncogene (You et al., 1989). Activation of the K-*ras* gene is frequently observed in chemically induced pulmonary neoplasms in rodents, and GC → AT transitions in the 12th codon were found more frequently in chemically induced pulmonary neoplasms than in spontaneously occurring neoplasms in the A/J strain of mice (Belinsky et al., 1989).

Although no studies have directly shown that tetranitromethane can react with DNA, results of mutagenicity studies indicate induction of base-pair substitutions and certain frame-shift mutations and are supportive of some type of interaction of the chemical with DNA. Alper and Ames (1975) reported that tetranitromethane did not increase the frequency of deletion mutants in *Salmonella typhimurium* LT2; however, NTP studies have shown that the chemical causes mutations in three strains of Salmonella (TA98, TA100, and TA1535) (Appendix H). NTP studies have also shown tetranitromethane capable of inducing chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells.

Tetranitromethane is known to be capable of nitrating hydroxyl groups of proteins, primarily of tyrosine residues, and has been used for years as a biochemical reagent for this purpose (Riordan and Vallee, 1972). Ptitsyn et al. (1979) showed modification of tyrosine residues in deoxyribonucleoproteins in vitro.

Given the strong induction of lung neoplasms by tetranitromethane, it is noteworthy that no primary nasal passage neoplasms were seen in the studies. Nonneoplastic lesions in the nasal passage were indicative of chronic irritation and included chronic inflammation of the nasal mucosa and hyperplasia and squamous metaplasia of the respiratory epithelium in both rats and mice. Evidence for inflammatory and regenerative lesions of the nasal cavity and for an absence of neoplasia has also been noted in other recent NTP inhalation studies with irritant chemicals. These include the studies with 2-chloroacetophenone (NTP, 1990a), CS2 (NTP, 1990b), *l*-epinephrine hydrochloride (NTP, 1990c), and vinyl toluene (NTP, 1990d).

A small number of male mice were evaluated after exposure to tetranitromethane for 1 year. The lesions observed in the respiratory tract corresponded to and were predictive for the types of lesions observed at the termination of the 2-year studies. However, another noteworthy finding, that 4 of the 10 mice exposed to 0.5 ppm had hepatocellular adenomas, was not subsequently correlated by the results after 2 years, in that this neoplasm occurred with a negative trend in male mice (Table C3).

Deichmann et al. (1963) showed that inhalation exposure of Swiss Webster mice to 0.2 ppm 3-nitro-3-hexene for up to 15 months resulted in increased incidences of lesions reported as adenomas and adenocarcinomas of the lung. Lewis et al. (1979) found hepatocellular carcinomas in 10/10 Sprague Dawley rats exposed for 6 months to 207 ppm 2-nitropropane; none was found in controls. Thus, it would appear that inhalation of several small nitrated aliphatic compounds presents a carcinogenic hazard. Further studies are required to extend these observations to

other nitrated compounds and other routes of exposure.

The experimental and tabulated data for the NTP Technical Report on tetranitromethane were examined for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations. As summarized in Appendix J, the audit revealed no major problems with the conduct of the studies or with collection and documentation of the experimental data. No discrepancies were found that influenced the final interpretation of the results of these studies.

Under the conditions of these 2-year inhalation studies, there was clear evidence of carcinogenic activity* of tetranitromethane for male and female F344/N rats and male and female B6C3F1 mice, based on increased incidences of alveolar/bronchiolar neoplasms in both species and squamous cell carcinomas of the lung in rats.

Chronic inflammation of the nasal mucosa was related to exposure in rats and female mice, and hyperplasia and squamous metaplasia of the respiratory epithelium were increased in exposed male rats.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 9.

V. REFERENCES

V. REFERENCES

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	2 ppm	5 ppm
DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Early deaths			
Moribund sacrifice	27	25	39
Natural death	5	8	7
Survivors			
Terminal sacrifice	18	17	4
Animals examined microscopically	50	50	50
ALIMENTARY SYSTEM			
Intestine large, cecum	(45)	(49)	(49)
Intestine large, colon	(49)	(47)	(49)
Intestine large, rectum	(46)	(48)	(48)
Intestine small, duodenum	(48)	(49)	(48)
Adenocarcinoma		1 (2%)	
Intestine small, ileum	(46)	(45)	(46)
Peyer's patch, fibrous histiocytoma		1 (2%)	
Intestine small, jejunum	(47)	(42)	(45)
Adenocarcinoma	1 (2%)		
Leiomyosarcoma	1 (2%)		
Polyp adenomatous			1 (2%)
Liver	(50)	(50)	(50)
Adenocarcinoma, metastatic, multiple, intestine small	1 (2%)		
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung		1 (2%)	
Fibrous histiocytoma		1 (2%)	
Hepatocellular carcinoma	1 (2%)		
Hepatocellular adenoma		1 (2%)	
Histiocytic sarcoma		1 (2%)	
Neoplastic nodule		1 (2%)	
Mesentery	(4)	(7)	(6)
Hemangioma	1 (25%)		
Histiocytic sarcoma		1 (14%)	
Pancreas	(49)	(48)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			6 (12%)
Histiocytic sarcoma		1 (2%)	
Acinus, adenoma			2 (4%)
Stomach, forestomach	(49)	(49)	(50)
Histiocytic sarcoma		1 (2%)	
Papilloma squamous		1 (2%)	
Tongue	(2)	(1)	
Squamous cell carcinoma	1 (50%)		
CARDIOVASCULAR SYSTEM			
Blood vessel	(2)	(3)	(2)
Aorta, adventitia, alveolar/bronchiolar carcinoma, metastatic, lung			1 (50%)
Heart	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		3 (6%)	5 (10%)
Fibrous histiocytoma		1 (2%)	
Squamous cell carcinoma, metastatic, lung			2 (4%)
Epicardium, carcinoma, metastatic, uncertain primary site		1 (2%)	
Epicardium, histiocytic sarcoma		1 (2%)	

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(50)	(50)
Capsule, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Adrenal gland, cortex	(50)	(49)	(48)
Adenoma	1 (2%)	1 (2%)	
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	3 (6%)
Medulla, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	1 (2%)
Adrenal gland, medulla	(46)	(47)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung			2 (4%)
Neuroblastoma benign			1 (2%)
Pheochromocytoma malignant	1 (2%)	2 (4%)	1 (2%)
Pheochromocytoma benign	12 (26%)	14 (30%)	7 (15%)
Pheochromocytoma benign, multiple		1 (2%)	3 (6%)
Bilateral, pheochromocytoma benign		1 (2%)	
Islets, pancreatic	(49)	(48)	(50)
Adenoma	3 (6%)	5 (10%)	6 (12%)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Carcinoma	4 (8%)	3 (6%)	4 (8%)
Carcinoma, two	1 (2%)		
Parathyroid gland	(47)	(48)	(43)
Adenoma		1 (2%)	
Pituitary gland	(49)	(49)	(48)
Pars distalis, adenoma	27 (55%)	22 (45%)	16 (33%)
Pars distalis, adenoma, two	2 (4%)	2 (4%)	2 (4%)
Pars distalis, carcinoma		1 (2%)	
Pars intermedia, adenoma	1 (2%)		
Thyroid gland	(49)	(49)	(50)
C-cell, adenoma	3 (6%)	1 (2%)	3 (6%)
C-cell, carcinoma		1 (2%)	
Follicular cell, carcinoma	1 (2%)		1 (2%)
GENERAL BODY SYSTEM			
Tissue, NOS		(1)	(2)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (100%)	1 (50%)
Squamous cell carcinoma, metastatic, lung			1 (50%)
GENITAL SYSTEM			
Epididymis	(49)	(49)	(50)
Preputial gland	(49)	(48)	(49)
Carcinoma	3 (6%)	2 (4%)	1 (2%)
Squamous cell carcinoma		1 (2%)	
Prostate	(48)	(50)	(46)
Adenocarcinoma	1 (2%)		
Adenoma		1 (2%)	
Seminal vesicle	(46)	(47)	(49)
Testes	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	19 (38%)	23 (46%)	23 (46%)
Interstitial cell, adenoma	14 (28%)	15 (30%)	16 (32%)
HEMATOPOIETIC SYSTEM			
Bone marrow	(50)	(49)	(49)
Lymph node	(50)	(50)	(50)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung		3 (6%)	1 (2%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
HEMATOPOIETIC SYSTEM			
Lymph node (Continued)	(50)	(50)	(50)
Mediastinal, histiocytic sarcoma		1 (2%)	
Lymph node, mandibular	(48)	(44)	(46)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Fibrous histiocytoma		1 (2%)	
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Lymph node, mesenteric	(47)	(48)	(48)
Histiocytic sarcoma		1 (2%)	
Spleen	(50)	(49)	(50)
Hemangiosarcoma			1 (2%)
Thymus	(36)	(42)	(43)
Alveolar/bronchiolar carcinoma, metastatic, lung			4 (9%)
INTEGUMENTARY SYSTEM			
Mammary gland	(43)	(46)	(47)
Adenoma			1 (2%)
Fibroadenoma	1 (2%)		1 (2%)
Skin	(50)	(49)	(50)
Basal cell adenoma	1 (2%)	1 (2%)	
Basal cell carcinoma	1 (2%)		
Keratoacanthoma	2 (4%)	2 (4%)	
Papilloma squamous		1 (2%)	
Trichoepithelioma		1 (2%)	
Subcutaneous tissue, fibroma	4 (8%)	2 (4%)	3 (6%)
Subcutaneous tissue, lipoma		1 (2%)	1 (2%)
Subcutaneous tissue, sarcoma		1 (2%)	
MUSCULOSKELETAL SYSTEM			
Bone	(50)	(49)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Periosteum, femur, fibrous histiocytoma		1 (2%)	
Right, femur, osteosarcoma		1 (2%)	
Skeletal muscle	(1)	(1)	
Hindlimb, fibrous histiocytoma		1 (100%)	
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
Granular cell tumor benign		1 (2%)	
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	11 (22%)	11 (22%)
Alveolar/bronchiolar adenoma, multiple		2 (4%)	
Alveolar/bronchiolar carcinoma		18 (36%)	4 (8%)
Alveolar/bronchiolar carcinoma, multiple		4 (8%)	35 (70%)
Alveolar/bronchiolar carcinoma, two		4 (8%)	7 (14%)
Fibrous histiocytoma		1 (2%)	
Histiocytic sarcoma		1 (2%)	
Pheochromocytoma malignant, metastatic, adrenal gland	1 (2%)		
Sarcoma			1 (2%)
Squamous cell carcinoma		1 (2%)	14 (28%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
RESPIRATORY SYSTEM			
Lung (Continued)	(50)	(50)	(50)
Squamous cell carcinoma, two			5 (10%)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Pleura, mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Nose	(48)	(49)	(50)
SPECIAL SENSES SYSTEM			
Zymbal gland	(1)	(2)	(1)
Adenoma			1 (100%)
Carcinoma	1 (100%)		
Papilloma squamous		2 (100%)	
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	5 (10%)
Squamous cell carcinoma, metastatic, lung			2 (4%)
Bilateral, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Capsule, histiocytic sarcoma		1 (2%)	
Ureter	(1)		(1)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (100%)
Urinary bladder	(49)	(49)	(48)
SYSTEMIC LESIONS			
Multiple organs	*(50)	*(50)	*(50)
Histiocytic sarcoma		1 (2%)	
Leukemia mononuclear	27 (54%)	26 (52%)	22 (44%)
Lymphoma malignant	1 (2%)		
Mesothelioma malignant	3 (6%)	2 (4%)	5 (10%)
TUMOR SUMMARY			
Total animals with primary neoplasms **	48	49	50
Total primary neoplasms	140	190	199
Total animals with benign neoplasms	47	46	49
Total benign neoplasms	92	114	98
Total animals with malignant neoplasms	38	43	50
Total malignant neoplasms	48	76	101
Total animals with secondary neoplasms ***	2	6	20
Total secondary neoplasms	2	13	42
Total animals with malignant neoplasms--uncertain primary site		1	

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: CHAMBER CONTROL

DAYS ON STUDY	294	401	422	483	492	545	546	563	569	604	618	618	644	644	644	644	646	649	653	658	660	661	668	671	686
CARCASS ID	05551	08111	05311	09711	09911	06771	08861	05221	08891	09411	07211	07551	05561	07331	08221	05881	08331	06111	09961	05441	08871	08441	07771	09211	06331
ALIMENTARY SYSTEM																									
Esophagus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																					X				
Leiomyosarcoma																									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, multiple, intestine small																					X				
Hepatocellular carcinoma																									
Mesentery							+					+										+			
Hemangioma																						X			
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
Tongue																									
Squamous cell carcinoma																									
Tooth																									+
CARDIOVASCULAR SYSTEM																									
Blood vessel																					+				
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X													
Adrenal gland, medulla	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M
Pheochromocytoma malignant																									
Pheochromocytoma benign				X									X								X	X	X		
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X																
Carcinoma							X											X							
Carcinoma, two																								X	
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X				X		X	X	X	X		X	X				X			X	X	X	
Pars distalis, adenoma, two																X				X					
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma											X				X										
Follicular cell, carcinoma														X											
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Coagulating gland																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																				X					
Prostate	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Seminal vesicle	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma										X	X						X	X			X				
Interstitial cell, adenoma				X	X			X				X				X			X						

+: Tissue examined microscopically
: Not examined
−: Present but not examined microscopically
I: Insufficient tissue

M: Missing
A: Autolysis precludes examination
X: Incidence of listed morphology

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: CHAMBER CONTROL (Continued)

DAYS ON STUDY	694	707	708	720	723	723	724	727	727	727	727	727	727	727	727	727	727	727	728	728	728	728	728	729	729	TOTAL:
CARCASS ID	09331	05991	06441	09881	06551	07411	05711	05111	06001	06221	06661	06681	06991	08881	09901	09951	09991	10101	07001	07111	07661	07881	07991	08001	08511	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	A	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	A	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenocarcinoma																										1
Leiomyosarcoma								X																		1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, multiple, intestine small																										1
Hepatocellular carcinoma			X																							1
Mesentery															+											4
Hemangioma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue		+										+														2
Squamous cell carcinoma		X																								1
Tooth																										1
CARDIOVASCULAR SYSTEM																										
Blood vessel																				+						2
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pheochromocytoma malignant															X											1
Pheochromocytoma benign						X					X		X			X	X						X	X		12
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma								X									X									3
Carcinoma							X																		X	4
Carcinoma, two																										1
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X	X	X	X		X		X	X	Y	X				X		X		X			X	X		X	27
Pars distalis, adenoma, two																										2
Pars intermedia, adenoma						X																				1
Thyroid gland	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma												X														3
Follicular cell, carcinoma																										1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Coagulating gland			+																							1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	49
Carcinoma																			X					X		3
Prostate	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenocarcinoma																			X							1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma						X	X		X		X	X	X	X		X	X	X	X	X	X			X		19
Interstitial cell, adenoma			X	X	X			X		X					X								X		X	14

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: CHAMBER CONTROL (Continued)

DAYS ON STUDY	294	401	422	483	492	545	546	563	569	604	618	618	644	644	644	646	646	649	653	658	660	661	668	671	686
CARCASS ID	05551	08111	05331	09971	09111	06771	08861	05521	08991	09441	07221	07551	05561	07331	08221	05881	08331	06111	09661	05441	08871	08841	07771	09921	06331
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	A	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
INTEGUMENTARY SYSTEM																									
Mammary gland	M	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																				X					
Basal cell carcinoma																			X						
Keratoacanthoma											X				X										
Subcutaneous tissue, fibroma		X																							
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	−	+
Skeletal muscle							+																		
NERVOUS SYSTEM																									
Brain	+	+	−	+	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Larynx	M	M	M	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Pheochromocytoma malignant, metastatic, adrenal gland																									
Nose	A	+	−	−	+	+	+	+	+	+	+	+	+	+	+	M	+	−	+	+	+	+	+	+	+
Trachea	+	+	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Eye			+																						
Zymbal gland														+											
Carcinoma														X											
URINARY SYSTEM																									
Kidney	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ureter																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+
Leukemia mononuclear			X	X	X	X		X	X		X	X	X			X		X	X		X	X			
Lymphoma malignant																	X								
Mesothelioma malignant												X								X					

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: CHAMBER CONTROL (Continued)

DAYS ON STUDY	694	707	708	720	723	723	724	727	727	727	727	727	727	727	727	727	727	727	728	728	728	728	729	729	TOTAL:
CARCASS ID	09331	05991	06411	09811	06511	07411	05711	05111	06011	06211	06611	06811	06911	08811	09011	09511	09911	10101	07011	07111	07611	07811	07911	08011	08511	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	M	+	+	+	M	+	+	M	M	M	M	+	+	+	+	+		+	+	+	M	+	+	M	36
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Fibroadenoma	X																									1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																										1
Basal cell carcinoma																										1
Keratoacanthoma																										2
Subcutaneous tissue, fibroma		X										X		X												4
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
RESPIRATORY SYSTEM																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																X										1
Pheochromocytoma malignant, metastatic, adrenal gland														X												1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Eye																										1
Zymbal gland																										1
Carcinoma																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ureter																			+							1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	49
SYSTEMIC LESIONS																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X				X			X	X	X	X	X	X	X	X					X	X	X				27
Lymphoma malignant																										1
Mesothelioma malignant											X															3

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: 2 ppm

DAYS ON STUDY	353	486	533	535	535	541	547	556	560	591	598	605	619	619	633	633	659	661	663	674	675	755	755	755	811	867
CARCASS ID	29411	25511	29711	27711	29611	27311	27111	25411	25911	29111	26711	26611	26511	29811	26911	27411	27211	28611	27511	28111	26411	28001	29911	28711	25111
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																			X							
Intestine small, ileum	A	+	A	+	+	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peyer's patch, fibrous histiocytoma																										
Intestine small, jejunum	A	+	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung																										
Fibrous histiocytoma																										
Hepatocellular adenoma																										
Histiocytic sarcoma																								X		
Neoplastic nodule											X															
Mesentery		+							+		+											+	+	+		
Histiocytic sarcoma																								X		
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Histiocytic sarcoma																								X		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Histiocytic sarcoma																								X		
Papilloma squamous																	X									
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	
Tongue																										
CARDIOVASCULAR SYSTEM																										
Blood vessel																						+				
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar carcinoma, metastatic, lung			X														X									
Fibrous histiocytoma																										
Epicardium, carcinoma, metastatic, uncertain primary site																										
Epicardium, histiocytic sarcoma																								X		
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adrenal gland, cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma																										
Alveolar/bronchiolar carcinoma, metastatic, lung																						X				
Medulla, alveolar/bronchiolar carcinoma, metastatic, lung																	X									
Adrenal gland, medulla	A	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+		+	+	+	+	+	+	+	+	
Pheochromocytoma malignant																										
Pheochromocytoma benign						X					X				X							X		X		
Pheochromocytoma benign, multiple																				X						
Bilateral, pheochromocytoma benign																										
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma															X			X								
Carcinoma					X																		X			
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Adenoma																			X							
Pituitary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pars distalis, adenoma										X	X	X	X			X	X		X		X	X				X
Pars distalis, adenoma, two																										
Pars distalis, carcinoma																										
Thyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
C-cell, adenoma																										
C-cell, carcinoma																										
GENERAL BODY SYSTEM																										
Tissue, NOS																	+									
Alveolar/bronchiolar carcinoma, metastatic, lung																	X									
GENITAL SYSTEM																										
Coagulating gland																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Preputial gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Carcinoma								X																		
Squamous cell carcinoma																										
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma																										
Seminal vesicle	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Bilateral, interstitial cell, adenoma				X			X							X	X				X		X		X	X	X
Interstitial cell, adenoma						X			X	X							X	X				X				

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 2 ppm (Continued)

DAYS ON STUDY	6 8 7	6 8 8	6 9 2	6 9 9	7 0 7	7 0 8	7 2 4	7 2 6	7 2 7	7 2 7	7 2 7	7 2 7	7 2 7	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	TOTAL:
CARCASS ID	2 5 7 1	2 6 3 1	2 6 1 1	2 8 4 1	2 8 2 1	2 7 0 1	3 0 0 1	2 8 5 1	2 5 2 1	2 5 3 1	2 5 6 1	2 5 8 1	2 6 0 1	2 6 2 1	2 6 8 1	2 7 6 1	2 7 8 1	2 7 9 1	2 8 3 1	2 8 8 1	2 8 9 1	2 9 0 1	2 9 2 1	2 9 3 1	2 9 5 1	TISSUES TUMORS
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma																									1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	45
Peyer's patch, fibrous histiocytoma					X																				1
Intestine small, jejunum	+	+	+	A	+	+	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	42
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung			X																						1
Fibrous histiocytoma					X																				1
Hepatocellular adenoma				X																					1
Histiocytic sarcoma																									1
Neoplastic nodule																									1
Mesentery																					+				7
Histiocytic sarcoma																									1
Pancreas	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																									1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																									1
Papilloma squamous																									1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	47
Tongue																				+					1
CARDIOVASCULAR SYSTEM																									
Blood vessel												+												+	3
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung			X																						3
Fibrous histiocytoma					X																				1
Epicardium, carcinoma, metastatic, uncertain primary site																			X						1
Epicardium, histiocytic sarcoma																									1
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																				X					1
Alveolar/bronchiolar carcinoma, metastatic, lung																									1
Medulla, alveolar/bronchiolar carcinoma, metastatic, lung																									1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pheochromocytoma malignant				X																				X	2
Pheochromocytoma benign						X		X				X	X				X	X			X			X	14
Pheochromocytoma benign, multiple																									1
Bilateral, pheochromocytoma benign							X																		1
Islets, pancreatic	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma							X															X	X		5
Carcinoma		X																							3
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																									1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X	X					X	X				X	X						X		X	X	X	X	X	22
Pars distalis, adenoma, two											X						X								2
Pars distalis, carcinoma																X									1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma																		X							1
C-cell, carcinoma												X													1
GENERAL BODY SYSTEM																									
Tissue, NOS																									1
Alveolar/bronchiolar carcinoma, metastatic, lung																									1
GENITAL SYSTEM																									
Coagulating gland		+																							1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	49
Preputial gland	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																			X						2
Squamous cell carcinoma																X									1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																		X							1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	47
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma			X		X	X	X		X	X		X			X	X		X		X	X	X		X	23
Interstitial cell, adenoma	X			X				X			X		X	X			X						X		15

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 2 ppm (Continued)

DAYS ON STUDY	353	486	533	535	535	541	547	556	560	591	598	605	619	619	633	633	659	661	663	674	675	675	675	681	687
CARCASS ID	29411	25511	29711	27711	29611	27311	27111	25411	25911	29111	26711	26611	26511	29811	26911	27411	27211	28611	27511	28114	26411	28001	29911	28711	25111
HEMATOPOIETIC SYSTEM																									
Blood																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung			X															X							
Mediastinal, histiocytic sarcoma																								X	
Lymph node, mandibular	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma																									
Lymph node, *mesenteric*	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								X	
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus		M	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	M	+	+	+	+
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	M	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Keratoacanthoma																									
Papilloma squamous																									
Trichoepithelioma																									
Subcutaneous tissue, fibroma																	X								
Subcutaneous tissue, lipoma																									
Subcutaneous tissue, sarcoma				X																					
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Periosteum, femur, fibrous histiocytoma																									
Right, femur, osteosarcoma																									
Skeletal muscle																									
Hindlimb, fibrous histiocytoma																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor benign																									
RESPIRATORY SYSTEM																									
Larynx	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X	X						X										X				X
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma								X	X	X		X				X	X			X				X	
Alveolar/bronchiolar carcinoma, multiple			X															X							
Alveolar/bronchiolar carcinoma, two															X							X			
Fibrous histiocytoma																									
Histiocytic sarcoma																								X	
Squamous cell carcinoma																									
Nose	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Eye			+			A					+												+		
Harderian gland						+																			
Lacrimal gland																									
Zymbal gland																						+			
Papilloma squamous																						X			
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																		X							
Bilateral, alveolar/bronchiolar carcinoma, metastatic, lung																	X								
Capsule, histiocytic sarcoma																								X	
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								X	
Leukemia mononuclear		X					X	X		X				X	X	X				X	X	X			X
Mesothelioma malignant									X																

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 2 ppm (Continued)

DAYS ON STUDY	6 8 7	6 8 8	6 9 2	6 9 9	7 0 7	7 0 8	7 2 4	7 2 6	7 2 7	7 2 7	7 2 7	7 2 7	7 2 7	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	TOTAL:
CARCASS ID	2 5 7 1	2 6 3 1	2 6 1 1	2 8 4 1	2 8 2 1	2 7 0 1	3 0 0 1	2 8 5 1	2 5 2 1	2 5 3 1	2 5 6 1	2 5 8 1	2 6 0 1	2 6 2 1	2 6 8 1	2 7 6 1	2 7 8 1	2 7 9 1	2 8 3 1	2 8 8 1	2 8 9 1	2 9 0 1	2 9 2 1	2 9 3 1	2 9 5 1	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Blood								+																		1
Bone marrow	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung			X																							3
Mediastinal, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	M	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	44
Fibrous histiocytoma					X																					1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	M	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
INTEGUMENTARY SYSTEM																										
Mammary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Basal cell adenoma																				X						1
Keratoacanthoma			X				X																			2
Papilloma squamous																	X									1
Trichoepithelioma																			X							1
Subcutaneous tissue, fibroma						X																				2
Subcutaneous tissue, lipoma							X																			1
Subcutaneous tissue, sarcoma																										1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Periosteum, femur, fibrous histiocytoma					X																					1
Right, femur, osteosarcoma																						X				1
Skeletal muscle					+																					1
Hindlimb, fibrous histiocytoma					X																					1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granular cell tumor benign																					X					1
RESPIRATORY SYSTEM																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma								X	X			X		X					X	X						11
Alveolar/bronchiolar adenoma, multiple										X			X													2
Alveolar/bronchiolar carcinoma					X	X			X	X	X		X		X			X	X				X			18
Alveolar/bronchiolar carcinoma, multiple			X									X														4
Alveolar/bronchiolar carcinoma, two							X							X												4
Fibrous histiocytoma					X																					1
Histiocytic sarcoma																										1
Squamous cell carcinoma																									X	1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Eye																										3
Harderian gland																										1
Lacrimal gland																				+						1
Zymbal gland																+										2
Papilloma squamous																X										2
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Bilateral, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Capsule, histiocytic sarcoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SYSTEMIC LESIONS																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear	X	X				X	X				X	X	X	X		X	X	X	X	X			X		X	26
Mesothelioma malignant	X																									2

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: 5 ppm

DAYS ON STUDY	490	500	506	513	518	521	532	541	546	547	547	548	548	548	549	562	562	573	576	590	601	601	604	605	609
CARCASS ID	18301	15611	16411	16881	18001	19111	19011	16511	17211	15811	17311	17411	19911	20012	15531	16611	17611	16111	18841	15911	17511	19881	19961	19711	16011
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	M	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A
Polyp adenomatous				X																					
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery							+				+	+		+	+										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Acinus, adenoma			X																						
Salivary glands	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARDIOVASCULAR SYSTEM																									
Blood vessel														+											
Aorta, adventitia, alveolar/bronchiolar carcinoma, metastatic, lung																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Squamous cell carcinoma, metastatic, lung											X														
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, alveolar/bronchiolar carcinoma, metastatic, lung																									
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																								X	
Medulla, alveolar/bronchiolar carcinoma, metastatic, lung			X																						
Adrenal gland, medulla	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																									X
Neuroblastoma benign								X																	
Pheochromocytoma malignant														X											
Pheochromocytoma benign																						X			
Pheochromocytoma benign, multiple														X											
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma						X							X										X		
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Carcinoma		X																							
Parathyroid gland	+	M	+	+	+	+	+	M	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	M
Pituitary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Pars distalis, adenoma						X	X			X		X		X	X			X							
Pars distalis, adenoma, two																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
Follicular cell, carcinoma													X												
GENERAL BODY SYSTEM																									
Tissue, NOS																									
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Squamous cell carcinoma, metastatic, lung																									
GENITAL SYSTEM																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																								X	
Prostate	+	+	M	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	M	+	M	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Testes	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X					X	X								X			X	X	X	X	X	X	X
Interstitial cell, adenoma									X	X	X	X	X					X	X	X					

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 5 ppm (Continued)

DAYS ON STUDY	610	618	629	632	632	638	653	653	657	659	673	674	674	685	694	696	698	706	708	710	723	728	728	728	728	TOTAL:
CARCASS ID	18111	18211	15111	16911	17911	19411	18511	18811	15511	16311	17811	15711	18711	16711	17011	19311	18611	17711	15411	19511	17111	15211	16211	18911	19211	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		A	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		A	+	+	M	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+			+	+	+	+	+	+	+	45
Polyp adenomatous																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery									+																	6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung						X										X		X		X				X		6
Acinus, adenoma																				X						2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CARDIOVASCULAR SYSTEM																										
Blood vessel						+																				2
Aorta, adventitia, alveolar/bronchiolar carcinoma, metastatic, lung						X																				1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung			X			X							X						X		X					5
Squamous cell carcinoma, metastatic, lung																X										2
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, alveolar/bronchiolar carcinoma, metastatic, lung																								X		1
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar carcinoma, metastatic, lung						X														X						3
Medulla, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	47
Alveolar/bronchiolar carcinoma, metastatic, lung		X																								2
Neuroblastoma benign																										1
Pheochromocytoma malignant																										1
Pheochromocytoma benign										X		X	X	X				X		X						7
Pheochromocytoma benign, multiple																							X	X		3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X																X					X			6
Alveolar/bronchiolar carcinoma, metastatic, lung														X												1
Carcinoma								X		X						X										4
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	43
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma	X	X			X								X	X	X	X	X								X	16
Pars distalis, adenoma, two											X	X														2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma			X			X						X														3
Follicular cell, carcinoma																										1
GENERAL BODY SYSTEM																										
Tissue, NOS			+													+										2
Alveolar/bronchiolar carcinoma, metastatic, lung			X																							1
Squamous cell carcinoma, metastatic, lung																X										1
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma			X	X		X	X	X	X	X									X	X	X	X	X	X	X	23
Interstitial cell, adenoma		X			X						X		X	X	X		X	X								16

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 5 ppm (Continued)

DAYS ON STUDY	497	500	506	513	518	521	532	541	546	547	547	548	548	548	549	562	562	573	576	590	601	601	604	605	609
CARCASS ID	18311	15611	16411	16811	18011	19111	19011	16511	17211	15811	17311	17411	19911	20011	15311	16611	17611	16111	18411	15911	17511	19811	19961	19711	16011
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	M	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X															X						
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Adenoma																									
Fibroadenoma												X													
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma			X						X																
Subcutaneous tissue, lipoma																X									
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma	X	X				X			X								X		X						X
Alveolar/bronchiolar carcinoma																	X			X	X	X			
Alveolar/bronchiolar carcinoma, multiple		X	X	X	X				X		X			X		X		X	X				X	X	X
Alveolar/bronchiolar carcinoma, two	X					X	X	X					X												
Sarcoma										X															
Squamous cell carcinoma					X					X	X	X			X				X						
Squamous cell carcinoma, two																				X			X	X	
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																									
Pleura, mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																						X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Ear																									
Eye			+			+																		+	
Zymbal gland																+									
Adenoma																X									
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																	X					X			X
Squamous cell carcinoma, metastatic, lung											X														
Ureter																									
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Urinary bladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X					X	X	X	X						X			X	X		X	X	X	X	X
Mesothelioma malignant												X			X			X		X					

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: 5 ppm (Continued)

DAYS ON STUDY	610	618	629	632	632	638	653	653	657	659	673	674	674	685	694	696	698	706	708	710	723	728	728	728	728	TOTAL:
CARCASS ID	18111	18211	15111	16911	17911	19411	18511	18811	15511	16311	17811	15711	18711	16711	17011	19311	18611	17711	15411	19511	17111	15211	16211	18911	19211	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung													X													1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	46
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																					X					1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																				X						1
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	43
Alveolar/bronchiolar carcinoma, metastatic, lung						X						X														4
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	47
Adenoma																				X						1
Fibroadenoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																									X	3
Subcutaneous tissue, lipoma																										1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung																				X						1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
RESPIRATORY SYSTEM																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X	X	X	X																						11
Alveolar/bronchiolar carcinoma																										4
Alveolar/bronchiolar carcinoma, multiple		X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	35
Alveolar/bronchiolar carcinoma, two	X							X																		7
Sarcoma																										1
Squamous cell carcinoma				X					X				X	X		X	X		X						X	14
Squamous cell carcinoma, two								X													X					5
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung						X																				1
Pleura, mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Ear																+										1
Eye																				+						4
Zymbal gland																										1
Adenoma																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																					X			X		5
Squamous cell carcinoma, metastatic, lung																X										2
Ureter						+																				1
Alveolar/bronchiolar carcinoma, metastatic, lung						X																				1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SYSTEMIC LESIONS																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X					X	X	X		X							X	X				X			X	22
Mesothelioma malignant							X	X																		5

TABLE A3. ANALYSIS OF PRIMARY NEOPLASMS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	2 ppm	5 ppm
Adrenal Medulla: Pheochromocytoma			
Overall Rates (a)	12/46 (26%)	16/48 (33%)	10/48 (21%)
Adjusted Rates (b)	45.5%	58.3%	83.2%
Terminal Rates (c)	6/18 (33%)	7/17 (41%)	2/3 (67%)
Day of First Observation	483	541	548
Life Table Tests (d)	P=0.022	P=0.252	P=0.031
Logistic Regression Tests (d)	P=0.412	P=0.290	P=0.433
Cochran-Armitage Trend Test (d)	P=0.277N		
Fisher Exact Test (d)		P=0.294	P=0.360N
Adrenal Medulla: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (a)	13/46 (28%)	17/48 (35%)	10/48 (21%)
Adjusted Rates (b)	50.1%	60.2%	83.2%
Terminal Rates (c)	7/18 (39%)	7/17 (41%)	2/3 (67%)
Day of First Observation	483	541	548
Life Table Tests (d)	P=0.029	P=0.253	P=0.037
Logistic Regression Tests (d)	P=0.475	P=0.294	P=0.493
Cochran-Armitage Trend Test (d)	P=0.203N		
Fisher Exact Test (d)		P=0.301	P=0.275N
Preputial Gland: Carcinoma			
Overall Rates (a)	3/49 (6%)	2/48 (4%)	1/49 (2%)
Adjusted Rates (b)	14.6%	8.1%	3.7%
Terminal Rates (c)	2/17 (12%)	1/17 (6%)	0/4 (0%)
Day of First Observation	658	556	605
Life Table Tests (d)	P=0.523N	P=0.492N	P=0.667N
Logistic Regression Tests (d)	P=0.287N	P=0.504N	P=0.444N
Cochran-Armitage Trend Test (d)	P=0.239N		
Fisher Exact Test (d)		P=0.510N	P=0.309N
Pancreatic Islets: Adenoma			
Overall Rates (a)	3/49 (6%)	5/48 (10%)	6/50 (12%)
Adjusted Rates (b)	13.2%	21.2%	42.2%
Terminal Rates (c)	2/18 (11%)	2/17 (12%)	1/4 (25%)
Day of First Observation	569	633	521
Life Table Tests (d)	P=0.014	P=0.347	P=0.031
Logistic Regression Tests (d)	P=0.168	P=0.340	P=0.244
Cochran-Armitage Trend Test (d)	P=0.225		
Fisher Exact Test (d)		P=0.346	P=0.254
Pancreatic Islets: Carcinoma			
Overall Rates (a)	5/49 (10%)	3/48 (6%)	4/50 (8%)
Adjusted Rates (b)	18.3%	9.3%	21.7%
Terminal Rates (c)	1/18 (6%)	0/17 (0%)	0/4 (0%)
Day of First Observation	546	535	500
Life Table Tests (d)	P=0.373	P=0.345N	P=0.387
Logistic Regression Tests (d)	P=0.437N	P=0.371N	P=0.529N
Cochran-Armitage Trend Test (d)	P=0.457N		
Fisher Exact Test (d)		P=0.369N	P=0.487N
Pancreatic Islets: Adenoma or Carcinoma			
Overall Rates (a)	8/49 (16%)	8/48 (17%)	10/50 (20%)
Adjusted Rates (b)	29.7%	28.5%	54.8%
Terminal Rates (c)	3/18 (17%)	2/17 (12%)	1/4 (25%)
Day of First Observation	546	535	500
Life Table Tests (d)	P=0.024	P=0.597N	P=0.038
Logistic Regression Tests (d)	P=0.327	P=0.591	P=0.383
Cochran-Armitage Trend Test (d)	P=0.365		
Fisher Exact Test (d)		P=0.590	P=0.416

TABLE A3. ANALYSIS OF PRIMARY NEOPLASMS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	1/50 (2%)	13/50 (26%)	11/50 (22%)
Adjusted Rates (b)	5.6%	51.8%	30.4%
Terminal Rates (c)	1/18 (6%)	7/17 (41%)	0/4 (0%)
Day of First Observation	727	535	497
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Logistic Regression Tests (d)	P=0.015	P<0.001	P=0.005
Cochran-Armitage Trend Test (d)	P=0.012		
Fisher Exact Test (d)		P<0.001	P=0.002
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	0/50 (0%)	26/50 (52%)	46/50 (92%)
Adjusted Rates (b)	0.0%	76.0%	100.0%
Terminal Rates (c)	0/18 (0%)	10/17 (59%)	4/4 (100%)
Day of First Observation		533	497
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Logistic Regression Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P<0.001	P<0.001
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	1/50 (2%)	33/50 (66%)	46/50 (92%)
Adjusted Rates (b)	5.6%	83.2%	100.0%
Terminal Rates (c)	1/18 (6%)	11/17 (65%)	4/4 (100%)
Day of First Observation	727	533	497
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Logistic Regression Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P<0.001	P<0.001
Lung: Squamous Cell Carcinoma			
Overall Rates (a)	0/50 (0%)	1/50 (2%)	19/50 (38%)
Adjusted Rates (b)	0.0%	5.9%	77.1%
Terminal Rates (c)	0/18 (0%)	1/17 (6%)	1/4 (25%)
Day of First Observation		727	518
Life Table Tests (d)	P<0.001	P=0.489	P<0.001
Logistic Regression Tests (d)	P<0.001	P=0.489	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.500	P<0.001
Pituitary Gland/Pars Distalis: Adenoma			
Overall Rates (a)	29/49 (59%)	24/49 (49%)	18/48 (38%)
Adjusted Rates (b)	76.2%	74.9%	70.7%
Terminal Rates (c)	10/18 (56%)	10/17 (59%)	1/4 (25%)
Day of First Observation	422	591	521
Life Table Tests (d)	P=0.165	P=0.299N	P=0.223
Logistic Regression Tests (d)	P=0.062N	P=0.185N	P=0.051N
Cochran-Armitage Trend Test (d)	P=0.022N		
Fisher Exact Test (d)		P=0.209N	P=0.026N
Pituitary Gland/Pars Distalis: Adenoma or Carcinoma			
Overall Rates (a)	29/49 (59%)	25/49 (51%)	18/48 (38%)
Adjusted Rates (b)	76.2%	78.5%	70.7%
Terminal Rates (c)	10/18 (56%)	11/17 (65%)	1/4 (25%)
Day of First Observation	422	591	521
Life Table Tests (d)	P=0.154	P=0.356N	P=0.223
Logistic Regression Tests (d)	P=0.064N	P=0.243N	P=0.051N
Cochran-Armitage Trend Test (d)	P=0.021N		
Fisher Exact Test (d)		P=0.271N	P=0.026N

TABLE A3. ANALYSIS OF PRIMARY NEOPLASMS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
Subcutaneous Tissue: Fibroma			
Overall Rates (e)	4/50 (8%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	16.6%	7.8%	28.3%
Terminal Rates (c)	2/18 (11%)	0/17 (0%)	1/4 (25%)
Day of First Observation	401	659	506
Life Table Tests (d)	P=0.390	P=0.361N	P=0.425
Logistic Regression Tests (d)	P=0.442N	P=0.338N	P=0.463N
Cochran-Armitage Trend Test (d)	P=0.467N		
Fisher Exact Test (d)		P=0.339N	P=0.500N
Subcutaneous Tissue: Fibroma or Sarcoma			
Overall Rates (e)	4/50 (8%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	16.6%	9.8%	28.3%
Terminal Rates (c)	2/18 (11%)	0/17 (0%)	1/4 (25%)
Day of First Observation	401	535	506
Life Table Tests (d)	P=0.417	P=0.517N	P=0.425
Logistic Regression Tests (d)	P=0.401N	P=0.509N	P=0.463N
Cochran-Armitage Trend Test (d)	P=0.447N		
Fisher Exact Test (d)		P=0.500N	P=0.500N
Testis: Interstitial Cell Adenoma			
Overall Rates (a)	33/50 (66%)	38/50 (76%)	39/50 (78%)
Adjusted Rates (b)	96.8%	97.3%	100.0%
Terminal Rates (c)	17/18 (94%)	16/17 (94%)	4/4 (100%)
Day of First Observation	483	535	500
Life Table Tests (d)	P<0.001	P=0.216	P<0.001
Logistic Regression Tests (d)	P=0.010	P=0.180	P=0.020
Cochran-Armitage Trend Test (d)	P=0.124		
Fisher Exact Test (d)		P=0.189	P=0.133
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	3/49 (6%)	1/49 (2%)	3/50 (6%)
Adjusted Rates (b)	10.7%	5.9%	15.6%
Terminal Rates (c)	1/17 (6%)	1/17 (6%)	0/4 (0%)
Day of First Observation	618	727	629
Life Table Tests (d)	P=0.240	P=0.322N	P=0.321
Logistic Regression Tests (d)	P=0.504	P=0.297N	P=0.616
Cochran-Armitage Trend Test (d)	P=0.572		
Fisher Exact Test (d)		P=0.309N	P=0.651N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	3/49 (6%)	2/49 (4%)	3/50 (6%)
Adjusted Rates (b)	10.7%	11.8%	15.6%
Terminal Rates (c)	1/17 (6%)	2/17 (12%)	0/4 (0%)
Day of First Observation	618	727	629
Life Table Tests (d)	P=0.215	P=0.515N	P=0.321
Logistic Regression Tests (d)	P=0.483	P=0.490N	P=0.616
Cochran-Armitage Trend Test (d)	P=0.591		
Fisher Exact Test (d)		P=0.500N	P=0.651N
Hematopoietic System: Mononuclear Leukemia			
Overall Rates (e)	27/50 (54%)	26/50 (52%)	22/50 (44%)
Adjusted Rates (b)	75.5%	78.7%	79.8%
Terminal Rates (c)	11/18 (61%)	11/17 (65%)	2/4 (50%)
Day of First Observation	422	486	497
Life Table Tests (d)	P=0.032	P=0.534N	P=0.063
Logistic Regression Tests (d)	P=0.216N	P=0.492N	P=0.195N
Cochran-Armitage Trend Test (d)	P=0.180N		
Fisher Exact Test (d)		P=0.500N	P=0.212N

TABLE A3. ANALYSIS OF PRIMARY NEOPLASMS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
All Sites: Mesothelioma			
Overall Rates (e)	3/50 (6%)	2/50 (4%)	5/50 (10%)
Adjusted Rates (b)	10.9%	6.1%	18.2%
Terminal Rates (c)	1/18 (6%)	0/17 (0%)	0/4 (0%)
Day of First Observation	618	560	548
Life Table Tests (d)	P=0.085	P=0.500N	P=0.131
Logistic Regression Tests (d)	P=0.288	P=0.502N	P=0.375
Cochran-Armitage Trend Test (d)	P=0.253		
Fisher Exact Test (d)		P=0.500N	P=0.357
All Sites: Benign Tumors			
Overall Rates (e)	47/50 (94%)	46/50 (92%)	49/50 (98%)
Adjusted Rates (b)	100.0%	100.0%	100.0%
Terminal Rates (c)	18/18 (100%)	17/17 (100%)	4/4 (100%)
Day of First Observation	401	535	497
Life Table Tests (d)	P<0.001	P=0.551	P<0.001
Logistic Regression Tests (d)	P=0.242	P=0.325N	P=0.322
Cochran-Armitage Trend Test (d)	P=0.231		
Fisher Exact Test (d)		P=0.500N	P=0.309
All Sites: Malignant Tumors			
Overall Rates (e)	38/50 (76%)	43/50 (86%)	50/50 (100%)
Adjusted Rates (b)	89.6%	95.3%	100.0%
Terminal Rates (c)	14/18 (78%)	15/17 (88%)	4/4 (100%)
Day of First Observation	422	486	497
Life Table Tests (d)	P<0.001	P=0.269	P<0.001
Logistic Regression Tests (d)	P<0.001	P=0.164	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.154	P<0.001
All Sites: All Tumors			
Overall Rates (e)	48/50 (96%)	49/50 (98%)	50/50 (100%)
Adjusted Rates (b)	100.0%	100.0%	100.0%
Terminal Rates (c)	18/18 (100%)	17/17 (100%)	4/4 (100%)
Day of First Observation	401	486	497
Life Table Tests (d)	P<0.001	P=0.450	P<0.001
Logistic Regression Tests (d)	P=0.265	P=0.685	P=0.274
Cochran-Armitage Trend Test (d)	P=0.160		
Fisher Exact Test (d)		P=0.500	P=0.247

(a) Number of tumor-bearing animals/number of animals examined microscopically at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or a lower incidence in a dosed group than in controls is indicated by (N).
(e) Number of tumor-bearing animals/number of animals examined grossly at the site

TABLE A4a. HISTORICAL INCIDENCE OF ALVEOLAR/BRONCHIOLAR NEOPLASMS IN MALE F344/N RATS (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence for Chamber Controls in NTP Studies (b)			
Propylene oxide	0/50	2/50	2/50
Methyl methacrylate	0/49	1/49	1/49
Propylene	0/50	1/50	1/50
1,2-Epoxybutane	0/50	0/50	0/50
Dichloromethane	1/50	0/50	1/50
Tetrachloroethylene	1/50	0/50	1/50
Bromoethane	0/48	0/48	0/48
TOTAL	2/347 (0.6%)	4/347 (1.2%)	6/347 (1.7%)
SD (c)	0.98%	1.58%	1.38%
Range (d)			
High	1/50	2/50	2/50
Low	0/50	0/50	0/50
Overall Historical Incidence for Untreated Controls in NTP Studies			
TOTAL	26/1,593 (1.6%)	20/1,593 (1.3%)	44/1,593 (2.8%)
SD (c)	1.81%	1.89%	2.32%
Range (d)			
High	3/49	3/50	4/50
Low	0/50	0/50	0/50

(a) Data as of March 1, 1989, for studies of at least 104 weeks
(b) All inhalation studies included in the NTP historical data base were conducted at Battelle Pacific Northwest Laboratories.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE A4b. HISTORICAL INCIDENCE OF LUNG SQUAMOUS CELL NEOPLASMS IN MALE F344/N RATS (a)

Study	Incidence of Carcinomas in Controls
Historical Incidence for Chamber Controls in NTP Studies (b)	
Propylene oxide	0/50
Methyl methacrylate	0/49
Propylene	0/50
1,2-Epoxybutane	0/50
Dichloromethane	0/50
Tetrachloroethylene	0/50
Bromoethane	(c) 1/48
TOTAL	(c) 1/347 (0.3%)
SD (d)	0.79%
Range (e)	
High	1/48
Low	0/50
Overall Historical Incidence for Untreated Controls in NTP Studies	
TOTAL	(f) 3/1,593 (0.2%)
SD (d)	0.60%
Range (e)	
High	1/49
Low	0/50

(a) Data as of March 1, 1989, for studies of at least 104 weeks; no benign tumors have been observed
(b) All inhalation studies included in the NTP historical data base were conducted at Battelle Pacific Northwest Laboratories.
(c) Adenosquamous carcinoma
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.
(f) Includes one carcinoma, NOS

TABLE A4c. HISTORICAL INCIDENCE OF TESTICULAR NEOPLASMS IN MALE F344/N RATS (a)

Study	Incidence of Interstitial Cell Neoplasms in Controls
Historical Incidence for Chamber Controls in NTP Studies (b)	
Propylene oxide	29/49
Methyl methacrylate	35/50
Propylene	37/50
1,2-Epoxybutane	39/50
Dichloromethane	39/50
Tetrachloroethylene	35/50
Bromoethane	42/48
TOTAL	256/347 (73.8%)
SD (c)	8.81%
Range (d)	
High	42/48
Low	29/49
Overall Historical Incidence for Untreated Controls in NTP Studies	
TOTAL	1,401/1,582 (88.6%)
SD (c)	7.33%
Range (d)	
High	49/49
Low	32/50

(a) Data as of March 1, 1989, for studies of at least 104 weeks
(b) All inhalation studies included in the NTP historical data base were conducted at Battelle Pacific Northwest Laboratories.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	2 ppm	5 ppm
DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Early deaths			
Moribund sacrifice	27	25	39
Natural death	5	8	7
Survivors			
Terminal sacrifice	18	17	4
Animals examined microscopically	50	50	50
ALIMENTARY SYSTEM			
Intestine large	(50)	(50)	(50)
Edema	1 (2%)		
Serosa, hemorrhage, chronic, focal	1 (2%)		
Intestine large, cecum	(45)	(49)	(49)
Edema			1 (2%)
Inflammation, chronic active	1 (2%)		
Parasite metazoan	4 (9%)	2 (4%)	
Intestine large, colon	(49)	(47)	(49)
Parasite metazoan	3 (6%)	3 (6%)	8 (16%)
Muscularis, mineralization, multifocal			1 (2%)
Intestine large, rectum	(46)	(48)	(48)
Parasite metazoan	4 (9%)	3 (6%)	3 (6%)
Serosa, inflammation, chronic	1 (2%)		
Intestine small	(50)	(50)	(49)
Peyer's patch, hyperplasia		1 (2%)	
Intestine small, duodenum	(48)	(49)	(48)
Ectopic tissue			1 (2%)
Mucosa, inflammation, necrotizing, acute		1 (2%)	
Muscularis, hyperplasia, focal		1 (2%)	
Intestine small, ileum	(46)	(45)	(46)
Infiltration cellular, eosinophilic, histiocytic	1 (2%)		
Mucosa, atrophy, diffuse			1 (2%)
Peyer's patch, hyperplasia, lymphoid			1 (2%)
Intestine small, jejunum	(47)	(42)	(45)
Congestion			1 (2%)
Diverticulum		1 (2%)	
Peyer's patch, hyperplasia, lymphoid	1 (2%)		
Liver	(50)	(50)	(50)
Angiectasis, focal	3 (6%)	3 (6%)	2 (4%)
Angiectasis, multifocal	1 (2%)	2 (4%)	
Basophilic focus	6 (12%)	1 (2%)	4 (8%)
Basophilic focus, multiple	4 (8%)	5 (10%)	5 (10%)
Basophilic focus, two	1 (2%)	5 (10%)	6 (12%)
Clear cell focus	1 (2%)		
Congestion, diffuse	1 (2%)		2 (4%)
Cytomegaly, diffuse	1 (2%)		
Cytomegaly, multifocal	3 (6%)		
Cytoplasmic alteration, focal		2 (4%)	
Degeneration, ballooning, focal	3 (6%)	1 (2%)	3 (6%)
Degeneration, ballooning, multifocal	1 (2%)	2 (4%)	
Eosinophilic focus	1 (2%)	2 (4%)	
Fatty change, diffuse	2 (4%)	1 (2%)	1 (2%)
Fatty change, multifocal	8 (16%)	4 (8%)	3 (6%)
Fibrosis, focal	1 (2%)		
Granuloma, multifocal	6 (12%)	4 (8%)	3 (6%)
Hemorrhage, acute, multifocal		2 (4%)	
Hyperplasia, nodular, multifocal		2 (4%)	
Inflammation, subacute, multifocal		1 (2%)	
Mitotic alteration	2 (4%)	1 (2%)	1 (2%)
Necrosis, acute, focal		1 (2%)	
Necrosis, acute, multifocal	1 (2%)	5 (10%)	6 (12%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
ALIMENTARY SYSTEM			
Liver (Continued)	(50)	(50)	(50)
Necrosis, subacute, multifocal		4 (8%)	
Vacuolization cytoplasmic, diffuse		1 (2%)	
Vacuolization cytoplasmic, multifocal	3 (6%)		
Bile duct, hyperplasia, multifocal	29 (58%)	30 (60%)	31 (62%)
Centrilobular, atrophy, diffuse	2 (4%)	1 (2%)	1 (2%)
Centrilobular, atrophy, multifocal	4 (8%)	3 (6%)	6 (12%)
Centrilobular, congestion		1 (2%)	1 (2%)
Centrilobular, degeneration, diffuse			1 (2%)
Centrilobular, degeneration, multifocal			2 (4%)
Centrilobular, fatty change, diffuse	1 (2%)	2 (4%)	1 (2%)
Centrilobular, fatty change, multifocal	1 (2%)		1 (2%)
Centrilobular, necrosis, diffuse	1 (2%)	1 (2%)	
Centrilobular, necrosis, multifocal	1 (2%)	2 (4%)	
Median lobe, hepatodiaphragmatic nodule	2 (4%)	1 (2%)	3 (6%)
Periportal, cytomegaly, diffuse	1 (2%)		
Periportal, fatty change, multifocal	1 (2%)		
Portal, fibrosis, multifocal	2 (4%)	1 (2%)	5 (10%)
Portal, inflammation, chronic, multifocal	1 (2%)		
Portal, inflammation, granulomatous, multifocal	1 (2%)		
Serosa, inflammation, proliferative, chronic active, focal	1 (2%)		
Vein, thrombus	1 (2%)		2 (4%)
Mesentery	(4)	(7)	(6)
Accessory spleen		1 (14%)	
Infiltration cellular, lymphocytic, multifocal			1 (17%)
Inflammation, subacute			1 (17%)
Pigmentation	1 (25%)		
Artery, mineralization			1 (17%)
Artery, adventitia, inflammation, chronic active, multifocal		1 (14%)	
Fat, necrosis, focal	1 (25%)	2 (29%)	
Pancreas	(49)	(48)	(50)
Angiectasis, focal			1 (2%)
Fibrosis, focal			1 (2%)
Inflammation, focal		1 (2%)	
Pigmentation	1 (2%)		
Acinus, atrophy, diffuse		2 (4%)	
Acinus, atrophy, focal	1 (2%)	3 (6%)	1 (2%)
Acinus, atrophy, multifocal	18 (37%)	12 (25%)	18 (36%)
Acinus, hyperplasia, focal		2 (4%)	
Artery, adventitia, inflammation, chronic active, multifocal	1 (2%)	1 (2%)	
Salivary glands	(50)	(50)	(49)
Inflammation, chronic, focal	1 (2%)		
Duct, inflammation, chronic active, multifocal			1 (2%)
Duct, metaplasia, squamous, multifocal			1 (2%)
Parotid gland, inflammation, chronic active, diffuse	1 (2%)		
Stomach	(50)	(50)	(50)
Inflammation, chronic active		1 (2%)	
Stomach, forestomach	(49)	(49)	(50)
Erosion		1 (2%)	
Hemorrhage, multifocal		1 (2%)	
Hyperkeratosis, multifocal			1 (2%)
Inflammation, acute, focal	1 (2%)		1 (2%)
Inflammation, chronic	2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic active	3 (6%)	6 (12%)	8 (16%)
Ulcer	4 (8%)	2 (4%)	4 (8%)
Epithelium, hyperplasia, diffuse	5 (10%)	2 (4%)	1 (2%)
Epithelium, hyperplasia, focal	1 (2%)	3 (6%)	3 (6%)
Epithelium, hyperplasia, multifocal		1 (2%)	1 (2%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
ALIMENTARY SYSTEM			
Stomach, forestomach (Continued)	(49)	(49)	(50)
Mucosa, mineralization, multifocal		1 (2%)	
Muscularis, hyperplasia, focal	1 (2%)		
Stomach, glandular	(48)	(47)	(50)
Inflammation, chronic	1 (2%)		1 (2%)
Mucosa, mineralization		2 (4%)	1 (2%)
Mucosa, necrosis, acute, multifocal	1 (2%)		
Tongue	(2)	(1)	
Epithelium, hyperplasia, focal	1 (50%)		
Tooth	(1)		
Abscess	1 (100%)		
Developmental malformation	1 (100%)		
Gingiva, inflammation	1 (100%)		
CARDIOVASCULAR SYSTEM			
Blood vessel	(2)	(3)	(2)
Inflammation, necrotizing, chronic, focal	1 (50%)		
Mineralization, focal	1 (50%)		
Aorta, mineralization, multifocal		1 (33%)	
Artery, aneurysm		1 (33%)	
Artery, arteriosclerosis, multifocal		1 (33%)	
Intima, fibrosis, multifocal	1 (50%)		
Pulmonary artery, mineralization			1 (50%)
Heart	(50)	(50)	(50)
Cardiomyopathy	45 (90%)	43 (86%)	36 (72%)
Dilatation		1 (2%)	
Inflammation, chronic active, focal			1 (2%)
Mineralization			1 (2%)
Atrium left, thrombus	2 (4%)	3 (6%)	2 (4%)
Atrium right, dilatation		1 (2%)	2 (4%)
Epicardium, hyperplasia			3 (6%)
Epicardium, inflammation, chronic, focal			1 (2%)
Mitral valve, inflammation, chronic			1 (2%)
Mitral valve, thrombus		1 (2%)	
Myocardium, inflammation, chronic, focal	1 (2%)		
Ventricle left, dilatation	1 (2%)		
Ventricle left, hypertrophy, focal			1 (2%)
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(50)	(50)
Capsule, accessory adrenal cortical nodule	4 (8%)	5 (10%)	8 (16%)
Adrenal gland, cortex	(50)	(49)	(48)
Angiectasis, multifocal		1 (2%)	
Congestion		1 (2%)	1 (2%)
Degeneration, fatty, diffuse	3 (6%)	10 (20%)	14 (29%)
Degeneration, fatty, focal	10 (20%)	5 (10%)	2 (4%)
Degeneration, fatty, multifocal	3 (6%)	4 (8%)	7 (15%)
Degeneration, multifocal	1 (2%)		
Hemorrhage, acute, multifocal		1 (2%)	
Hyperplasia, focal	6 (12%)	4 (8%)	4 (8%)
Hyperplasia, multifocal	1 (2%)	5 (10%)	1 (2%)
Hypertrophy, focal	1 (2%)	2 (4%)	1 (2%)
Necrosis, acute, focal	1 (2%)	1 (2%)	
Necrosis, acute, multifocal		1 (2%)	
Adrenal gland, medulla	(46)	(47)	(47)
Hyperplasia, focal	7 (15%)	9 (19%)	4 (9%)
Hyperplasia, multifocal	9 (20%)	11 (23%)	13 (28%)
Necrosis, acute, focal	1 (2%)		
Islets, pancreatic	(49)	(48)	(50)
Hyperplasia			1 (2%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
ENDOCRINE SYSTEM			
Islets, pancreatic (Continued)	(49)	(48)	(50)
Hyperplasia, focal	2 (4%)	7 (15%)	2 (4%)
Hyperplasia, multifocal		1 (2%)	3 (6%)
Parathyroid gland	(47)	(48)	(43)
Hyperplasia, diffuse	3 (6%)	4 (8%)	1 (2%)
Hyperplasia, focal		1 (2%)	
Pituitary gland	(49)	(49)	(48)
Fibrosis, focal			1 (2%)
Necrosis, subacute			1 (2%)
Pars distalis, angiectasis	2 (4%)	3 (6%)	1 (2%)
Pars distalis, cyst	1 (2%)	3 (6%)	1 (2%)
Pars distalis, cyst, multiple	1 (2%)	1 (2%)	2 (4%)
Pars distalis, developmental malformation	1 (2%)	1 (2%)	
Pars distalis, hyperplasia	4 (8%)	1 (2%)	
Pars distalis, hyperplasia, focal	1 (2%)	3 (6%)	6 (13%)
Pars distalis, hyperplasia, multifocal			1 (2%)
Pars distalis, hypertrophy, focal		1 (2%)	1 (2%)
Pars distalis, hypertrophy, multifocal		1 (2%)	
Pars intermedia, angiectasis, multifocal			1 (2%)
Thyroid gland	(49)	(49)	(50)
C-cell, hyperplasia, focal	3 (6%)	2 (4%)	5 (10%)
C-cell, hyperplasia, multifocal	2 (4%)	6 (12%)	2 (4%)
Follicle, cyst		1 (2%)	
Follicle, cyst, multiple		1 (2%)	
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Coagulating gland	(1)	(1)	
Abscess, chronic, multiple		1 (100%)	
Hyperplasia		1 (100%)	
Inflammation, chronic active	1 (100%)		
Epididymis	(49)	(49)	(50)
Atrophy	2 (4%)		
Ectasia	1 (2%)		
Fibrosis	1 (2%)		
Serosa, granuloma, multifocal	1 (2%)		
Preputial gland	(49)	(48)	(49)
Abscess			1 (2%)
Atrophy			1 (2%)
Cyst			1 (2%)
Ectasia	3 (6%)	4 (8%)	7 (14%)
Foreign body		3 (6%)	
Hyperplasia	1 (2%)	1 (2%)	2 (4%)
Hyperplasia, squamous		1 (2%)	
Inflammation, chronic	1 (2%)		
Inflammation, chronic active	3 (6%)		
Inflammation, granulomatous	28 (57%)	28 (58%)	30 (61%)
Prostate	(48)	(50)	(46)
Abscess	1 (2%)	2 (4%)	1 (2%)
Ectasia, focal			1 (2%)
Hemorrhage, acute	1 (2%)		
Hyperplasia, focal	6 (13%)	3 (6%)	3 (7%)
Hyperplasia, multifocal	4 (8%)	5 (10%)	2 (4%)
Inflammation, chronic	1 (2%)		1 (2%)
Inflammation, chronic active	5 (10%)	1 (2%)	1 (2%)
Inflammation, suppurative	1 (2%)		
Inflammation, suppurative, chronic active	1 (2%)	1 (2%)	
Mineralization, multifocal		1 (2%)	

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
GENITAL SYSTEM (Continued)			
Seminal vesicle	(46)	(47)	(49)
Atrophy		3 (6%)	1 (2%)
Concretion	1 (2%)		
Ectasia	2 (4%)		
Hyperplasia	1 (2%)		
Testes	(50)	(50)	(50)
Mineralization, focal		2 (4%)	1 (2%)
Mineralization, multifocal	4 (8%)	3 (6%)	1 (2%)
Artery, inflammation, chronic	1 (2%)	1 (2%)	1 (2%)
Artery, inflammation, chronic active, multifocal	1 (2%)	3 (6%)	
Interstitial cell, hyperplasia	9 (18%)	16 (32%)	21 (42%)
Seminiferous tubule, atrophy	12 (24%)	9 (18%)	12 (24%)
Seminiferous tubule, degeneration	2 (4%)	1 (2%)	1 (2%)
Serosa, necrosis, focal	1 (2%)		
HEMATOPOIETIC SYSTEM			
Bone marrow	(50)	(49)	(49)
Hyperplasia	12 (24%)	13 (27%)	6 (12%)
Myelofibrosis, focal		1 (2%)	
Myeloid cell, hyperplasia			1 (2%)
Lymph node	(50)	(50)	(50)
Inguinal, hemorrhage, acute		1 (2%)	
Mediastinal, cyst			3 (6%)
Mediastinal, cyst, multiple			4 (8%)
Mediastinal, hemorrhage, acute	3 (6%)	7 (14%)	5 (10%)
Mediastinal, hemorrhage, subacute	2 (4%)	3 (6%)	4 (8%)
Mediastinal, hyperplasia			1 (2%)
Mediastinal, hyperplasia, re cell	1 (2%)		
Mediastinal, pigmentation			2 (4%)
Pancreatic, edema	1 (2%)		
Pancreatic, granuloma, multifocal		1 (2%)	
Pancreatic, hemorrhage, acute	1 (2%)		1 (2%)
Pancreatic, hyperplasia			1 (2%)
Renal, hemorrhage, acute			1 (2%)
Lymph node, mandibular	(48)	(44)	(46)
Hemorrhage, acute	1 (2%)	2 (5%)	2 (4%)
Hemorrhage, subacute			1 (2%)
Hyperplasia		2 (5%)	1 (2%)
Hyperplasia, plasma cell	7 (15%)	2 (5%)	4 (9%)
Inflammation, chronic active			2 (4%)
Lymph node, mesenteric	(47)	(48)	(48)
Cyst	1 (2%)		1 (2%)
Hemorrhage, acute	4 (9%)	10 (21%)	6 (13%)
Hemorrhage, subacute		1 (2%)	
Hyperplasia		1 (2%)	
Hyperplasia, lymphoid	1 (2%)		1 (2%)
Hyperplasia, re cell	1 (2%)	1 (2%)	2 (4%)
Inflammation, subacute			1 (2%)
Spleen	(50)	(49)	(50)
Congestion, acute			1 (2%)
Depletion lymphoid	1 (2%)	1 (2%)	1 (2%)
Fibrosis		1 (2%)	
Fibrosis, diffuse		1 (2%)	1 (2%)
Fibrosis, focal	5 (10%)	6 (12%)	1 (2%)
Fibrosis, multifocal	1 (2%)		
Hematopoietic cell proliferation	2 (4%)	4 (8%)	7 (14%)
Hemorrhage		1 (2%)	
Hyperplasia, re cell, focal	1 (2%)		
Infarct	1 (2%)	1 (2%)	
Necrosis, subacute, multifocal			1 (2%)
Pigmentation, hemosiderin	4 (8%)		1 (2%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
HEMATOPOIETIC SYSTEM			
Spleen (Continued)	(50)	(49)	(50)
Capsule, fibrosis, multifocal		1 (2%)	
Thymus	(36)	(42)	(43)
Atrophy		1 (2%)	
Ectopic parathyroid gland	1 (3%)	2 (5%)	2 (5%)
Hemorrhage, acute		2 (5%)	
Epithelial cell, hyperplasia	1 (3%)	6 (14%)	2 (5%)
INTEGUMENTARY SYSTEM			
Mammary gland	(43)	(46)	(47)
Ectasia, diffuse	4 (9%)	1 (2%)	1 (2%)
Ectasia, multifocal	11 (26%)	11 (24%)	8 (17%)
Hyperplasia, diffuse	2 (5%)	3 (7%)	5 (11%)
Artery, adventitia, inflammation, chronic active, focal		1 (2%)	
Skin	(50)	(49)	(50)
Cyst dermoid		2 (4%)	2 (4%)
Hyperkeratosis	1 (2%)		
Inflammation, suppurative, chronic active, focal			1 (2%)
Right, forelimb, hemorrhage, chronic, subacute, focal		1 (2%)	
Subcutaneous tissue, developmental malformation		1 (2%)	
Tail, inflammation, chronic			1 (2%)
Tail, subcutaneous tissue, inflammation, proliferative, chronic	1 (2%)		
Tail, epithelium, hyperplasia			1 (2%)
MUSCULOSKELETAL SYSTEM			
Bone	(50)	(49)	(49)
Fibrous osteodystrophy		2 (4%)	1 (2%)
Hyperostosis, focal		1 (2%)	
Necrosis, acute	1 (2%)		
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
Compression	14 (28%)	10 (20%)	3 (6%)
Hemorrhage, multifocal	1 (2%)	1 (2%)	4 (8%)
Hydrocephalus	6 (12%)	3 (6%)	1 (2%)
Necrosis, focal	1 (2%)		
RESPIRATORY SYSTEM			
Larynx	(47)	(49)	(50)
Foreign body	2 (4%)	1 (2%)	
Inflammation, acute			1 (2%)
Inflammation, chronic	4 (9%)	4 (8%)	2 (4%)
Inflammation, chronic active	9 (19%)	10 (20%)	4 (8%)
Epithelium, hyperplasia		2 (4%)	
Lung	(50)	(50)	(50)
Abscess			1 (2%)
Giant cell, multifocal		1 (2%)	
Hemorrhage, acute, focal			1 (2%)
Hemorrhage, acute, multifocal		2 (4%)	1 (2%)
Hemorrhage, subacute, multifocal		2 (4%)	
Infiltration cellular, histiocytic, focal		1 (2%)	
Infiltration cellular, histiocytic, multifocal		1 (2%)	1 (2%)
Inflammation, acute, multifocal	2 (4%)		
Metaplasia, osseous, focal	1 (2%)		

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
RESPIRATORY SYSTEM			
Lung (Continued)	(50)	(50)	(50)
Necrosis, acute, multifocal			1 (2%)
Pigmentation, hemosiderin, diffuse		1 (2%)	
Pigmentation, hemosiderin, multifocal		2 (4%)	
Alveolar epithelium, hyperplasia, focal	1 (2%)	7 (14%)	
Alveolar epithelium, hyperplasia, multifocal		37 (74%)	50 (100%)
Artery, thrombus		1 (2%)	
Artery, adventitia, mediastinum, inflammation, chronic active, multifocal		1 (2%)	
Artery, mediastinum, mineralization			1 (2%)
Bronchiole, hyperplasia	1 (2%)		
Bronchiole, hyperplasia, focal		2 (4%)	
Bronchiole, hyperplasia, multifocal		21 (42%)	45 (90%)
Bronchiole, metaplasia, squamous, focal		1 (2%)	
Bronchus, hyperplasia, focal		1 (2%)	
Bronchus, metaplasia, squamous			1 (2%)
Bronchus, alveolus, inflammation, chronic active, multifocal	1 (2%)		
Interstitium, fibrosis, multifocal		1 (2%)	
Interstitium, inflammation, chronic, diffuse		1 (2%)	
Interstitium, inflammation, chronic, multifocal	1 (2%)		1 (2%)
Interstitium, inflammation, chronic active, diffuse		1 (2%)	
Interstitium, inflammation, chronic active, multifocal	1 (2%)		
Interstitium, mineralization, multifocal		1 (2%)	1 (2%)
Mediastinum, angiectasis			1 (2%)
Mediastinum, cyst			1 (2%)
Mediastinum, pigmentation			1 (2%)
Peribronchiolar, perivascular, granuloma, multifocal	1 (2%)		
Nose	(48)	(49)	(50)
Foreign body	3 (6%)	10 (20%)	3 (6%)
Lumen, exudate			1 (2%)
Mucosa, inflammation, acute		1 (2%)	
Mucosa, inflammation, chronic	2 (4%)		
Mucosa, inflammation, chronic active	9 (19%)	16 (33%)	16 (32%)
Mucosa, inflammation, suppurative, chronic active	1 (2%)	4 (8%)	21 (42%)
Mucosa, thrombus, multifocal	2 (4%)	3 (6%)	3 (6%)
Nasolacrimal duct, exudate		1 (2%)	
Nasolacrimal duct, hyperplasia	1 (2%)		
Nasolacrimal duct, inflammation, chronic	2 (4%)	4 (8%)	1 (2%)
Nasolacrimal duct, inflammation, chronic active	1 (2%)	2 (4%)	2 (4%)
Nasolacrimal duct, inflammation, suppurative, acute	3 (6%)	2 (4%)	
Nasolacrimal duct, inflammation, suppurative, chronic active		1 (2%)	
Olfactory epithelium, atrophy			3 (6%)
Olfactory epithelium, metaplasia	3 (6%)		
Respiratory epithelium, hyperplasia	7 (15%)	15 (31%)	29 (58%)
Respiratory epithelium, metaplasia, squamous		1 (2%)	13 (26%)
Trachea	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	3 (6%)	
Inflammation, chronic active			1 (2%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
SPECIAL SENSES SYSTEM			
Ear			(1)
Bilateral, external ear, hyperkeratosis			1 (100%)
Eye	(1)	(3)	(4)
Bilateral, cornea, mineralization, multifocal	1 (100%)		
Lens, cataract		1 (33%)	2 (50%)
Retina, degeneration		2 (67%)	2 (50%)
Harderian gland		(1)	
Inflammation, chronic		1 (100%)	
Zymbal gland	(1)	(2)	(1)
Inflammation, chronic active			1 (100%)
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Cyst, two			1 (2%)
Hydronephrosis		1 (2%)	
Infarct		2 (4%)	1 (2%)
Infiltration cellular, lymphocytic, multifocal		1 (2%)	
Nephropathy, chronic	47 (94%)	47 (94%)	49 (98%)
Pigmentation, diffuse	4 (8%)	3 (6%)	3 (6%)
Pigmentation, multifocal	1 (2%)	3 (6%)	1 (2%)
Artery, thrombus		1 (2%)	
Bilateral, hydronephrosis	1 (2%)		
Cortex, cyst, multiple		2 (4%)	
Cortex, renal tubule, hyperplasia, atypical, focal	2 (4%)		1 (2%)
Pelvis, inflammation, acute, diffuse	1 (2%)		
Pelvis, transitional epithelium, hyperplasia	1 (2%)		
Proximal convoluted renal tubule, necrosis, acute			1 (2%)
Ureter	(1)		(1)
Inflammation, chronic	1 (100%)		
Urinary bladder	(49)	(49)	(48)
Inflammation, chronic, diffuse	1 (2%)		
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)
Transitional epithelium, hyperplasia, multifocal	1 (2%)		

APPENDIX B

SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	2 ppm	5 ppm
DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Early deaths			
Natural death	6	3	8
Moribund sacrifice	19	13	27
Survivors			
Terminal sacrifice	25	34	15
Animals examined microscopically	50	50	50
ALIMENTARY SYSTEM			
Intestine large, cecum	(49)	(48)	(48)
Intestine large, colon	(50)	(49)	(48)
Intestine large, rectum	(49)	(49)	(49)
Intestine small, duodenum	(49)	(49)	(49)
Leiomyoma			1 (2%)
Intestine small, ileum	(46)	(49)	(46)
Intestine small, jejunum	(44)	(47)	(43)
Liver	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Hepatocellular carcinoma			1 (2%)
Hepatocellular adenoma	1 (2%)	1 (2%)	
Sarcoma, metastatic, lung			1 (2%)
Mesentery	(4)	(6)	(8)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (13%)
Sarcoma, metastatic, uncertain primary site			1 (13%)
Sarcoma, metastatic, uterus			1 (13%)
Pancreas	(50)	(50)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung			3 (6%)
Mixed tumor malignant, metastatic, lung			1 (2%)
Sarcoma, metastatic, lung			1 (2%)
Salivary glands	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
CARDIOVASCULAR SYSTEM			
Heart	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			3 (6%)
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung			1 (2%)
Sarcoma, metastatic, multiple, lung			1 (2%)
Epicardium, carcinoma, metastatic, lung			1 (2%)
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Adrenal gland, cortex	(50)	(49)	(49)
Adenoma			3 (6%)
Alveolar/bronchiolar carcinoma, metastatic, lung			4 (8%)
Carcinoma			1 (2%)
Carcinoma, metastatic, lung			1 (2%)
Medulla, alveolar/bronchiolar carcinoma, metastatic, lung			2 (4%)

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
ENDOCRINE SYSTEM (Continued)			
Adrenal gland, medulla	(43)	(48)	(45)
Pheochromocytoma complex			1 (2%)
Pheochromocytoma benign	2 (5%)	3 (6%)	2 (4%)
Sarcoma, metastatic, lung			1 (2%)
Bilateral, pheochromocytoma benign		1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(49)
Carcinoma			1 (2%)
Pituitary gland	(50)	(48)	(50)
Pars distalis, adenoma	27 (54%)	27 (56%)	24 (48%)
Pars distalis, adenoma, two	1 (2%)	2 (4%)	1 (2%)
Pars distalis, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Pars distalis, carcinoma	1 (2%)	2 (4%)	
Pars intermedia, adenoma			1 (2%)
Thyroid gland	(49)	(50)	(50)
C-cell, adenoma	3 (6%)	6 (12%)	4 (8%)
C-cell, carcinoma	3 (6%)	2 (4%)	1 (2%)
Follicular cell, adenoma			1 (2%)
Follicular cell, carcinoma			1 (2%)
GENERAL BODY SYSTEM			
Tissue, NOS			(3)
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung			1 (33%)
Sarcoma, metastatic, uncertain primary site			1 (33%)
Squamous cell carcinoma, metastatic, lung			1 (33%)
GENITAL SYSTEM			
Clitoral gland	(47)	(48)	(44)
Adenoma	1 (2%)	4 (8%)	
Carcinoma	2 (4%)	1 (2%)	
Ovary	(50)	(50)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung			5 (10%)
Bilateral, carcinoma, metastatic, lung			1 (2%)
Bilateral, sarcoma, metastatic, lung			1 (2%)
Uterus	(50)	(50)	(50)
Deciduoma benign		1 (2%)	
Polyp stromal	9 (18%)	8 (16%)	5 (10%)
Polyp stromal, two		1 (2%)	
Sarcoma			2 (4%)
Sarcoma stromal		1 (2%)	
Bilateral, polyp stromal	2 (4%)	3 (6%)	
Vagina	(2)	(1)	
Schwannoma malignant, metastatic, urinary bladder		1 (100%)	
HEMATOPOIETIC SYSTEM			
Bone marrow	(48)	(50)	(50)
Lymph node	(50)	(50)	(50)
Sarcoma, metastatic, lung			1 (2%)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung			5 (10%)
Mediastinal, carcinoma, metastatic, lung			1 (2%)
Serosa, mediastinal, sarcoma, metastatic, uncertain primary site			1 (2%)
Lymph node, mandibular	(49)	(50)	(49)

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
HEMATOPOIETIC SYSTEM (Continued)			
Lymph node, mesenteric	(47)	(49)	(49)
Spleen	(50)	(50)	(50)
Squamous cell carcinoma, metastatic, lung			1 (2%)
Thymus	(48)	(46)	(42)
Alveolar/bronchiolar carcinoma, metastatic, lung			4 (10%)
INTEGUMENTARY SYSTEM			
Mammary gland	(48)	(50)	(50)
Adenocarcinoma		1 (2%)	
Adenoma			1 (2%)
Fibroadenoma	7 (15%)	11 (22%)	4 (8%)
Fibroadenoma, two	1 (2%)	1 (2%)	2 (4%)
Skin	(50)	(50)	(50)
Basal cell adenoma		1 (2%)	
Basosquamous tumor malignant		1 (2%)	
Keratoacanthoma	1 (2%)		1 (2%)
Papilloma squamous		2 (4%)	1 (2%)
Subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic			1 (2%)
Subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Subcutaneous tissue, fibroma		1 (2%)	1 (2%)
Subcutaneous tissue, fibrosarcoma	1 (2%)		
Subcutaneous tissue, sarcoma	1 (2%)		
Vulva, papilloma	1 (2%)		
MUSCULOSKELETAL SYSTEM			
None			
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
Astrocytoma benign	1 (2%)		
Astrocytoma malignant			2 (4%)
Cerebellum, meningioma malignant	1 (2%)		
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		6 (12%)	3 (6%)
Alveolar/bronchiolar carcinoma		11 (22%)	3 (6%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)	44 (88%)
Alveolar/bronchiolar carcinoma, two		7 (14%)	3 (6%)
Chordoma, metastatic, uncertain primary site		1 (2%)	
Mixed tumor malignant, multiple			1 (2%)
Osteosarcoma, metastatic, uncertain primary site			1 (2%)
Sarcoma, multiple			1 (2%)
Squamous cell carcinoma		1 (2%)	10 (20%)
Squamous cell carcinoma, multiple			2 (4%)
Mediastinum, carcinoma, metastatic, lung			1 (2%)
Mediastinum, squamous cell carcinoma, metastatic, lung			1 (2%)
Pleura, mediastinum, sarcoma, metastatic, uncertain primary site			1 (2%)

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
SPECIAL SENSES SYSTEM			
None			
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			7 (14%)
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung			2 (4%)
Carcinoma, metastatic, lung			1 (2%)
Osteosarcoma, metastatic, uncertain primary site			1 (2%)
Sarcoma, metastatic, lung			2 (4%)
Urinary bladder	(49)	(50)	(48)
Schwannoma malignant		1 (2%)	
Transitional epithelium, papilloma		1 (2%)	
SYSTEMIC LESIONS			
Multiple organs	*(50)	*(50)	*(50)
Leukemia			1 (2%)
Leukemia mononuclear	18 (36%)	10 (20%)	6 (12%)
TUMOR SUMMARY			
Total animals with primary neoplasms**	47	49	50
Total primary neoplasms	84	119	137
Total animals with benign neoplasms	37	42	37
Total benign neoplasms	57	80	56
Total animals with malignant neoplasms	24	31	50
Total malignant neoplasms	27	39	81
Total animals with secondary neoplasms***		2	20
Total secondary neoplasms		2	69
Total animals with malignant neoplasms--uncertain primary site		1	2

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: CHAMBER CONTROL

DAYS ON STUDY	0 0 8	2 5 1	4 5 4	4 9 1	5 1 0	5 1 3	5 2 0	5 2 5	5 4 8	5 9 0	5 9 5	6 0 2	6 0 6	6 2 9	6 3 8	6 4 4	6 4 6	6 5 7	6 5 8	6 5 9	6 5 9	6 7 3	6 7 4	6 9 3	7 2 5
CARCASS ID	4 9 1	4 3 1	2 0 1	1 7 1	0 5 1	4 5 1	3 5 1	3 8 1	1 6 1	1 2 1	2 6 1	0 7 1	4 6 1	0 1 1	3 6 1	0 2 1	2 8 1	3 4 1	4 2 1	1 3 1	4 8 1	4 0 1	1 5 1	5 0 1	1 4 1
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	+	+	+	+	A	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	A	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	M	A
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery																				+		+			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	M	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma								X			X	X	X			X		X			X		X	X	X
Pars distalis, adenoma, two																									
Pars distalis, carcinoma																	X								
Thyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																							X		
C-cell, carcinoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Clitoral gland	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal			X			X		X					X												
Bilateral, polyp stromal							X												X						
Vagina																		+			+				

+: Tissue examined microscopically
: Not examined
–: Present but not examined microscopically
I: Insufficient tissue

M: Missing
A: Autolysis precludes examination
X: Incidence of listed morphology

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: CHAMBER CONTROL (Continued)

DAYS ON STUDY	727	727	727	727	727	727	727	727	727	727	727	728	728	728	728	728	729	729	729	729	729	729	729	729	729	TOTAL:
CARCASS ID	03 1	04 1	06 1	08 1	09 1	10 1	37 1	39 1	41 1	44 1	47 1	11 1	18 1	19 1	21 1	22 1	23 1	24 1	25 1	27 1	29 1	30 1	31 1	32 1	33 1	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma			X																							1
Mesentery									+											+						4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Pheochromocytoma benign											X	X														2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X		X		X	X		X	X		X	X	X	X	X	X	X	X	X	X				X	27
Pars distalis, adenoma, two								X																		1
Pars distalis, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma					X																X					3
C-cell, carcinoma	X					X	X																			3
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma												X														1
Carcinoma	X																X									2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal						X				X	X	X										X				9
Bilateral, polyp stromal																										2
Vagina																										2

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: CHAMBER CONTROL (Continued)

DAYS ON STUDY	008	251	454	491	510	513	520	525	548	590	595	602	606	629	638	644	646	657	658	659	659	673	674	693	725
CARCASS ID	491	431	201	171	051	451	351	381	161	121	261	071	461	011	361	021	281	341	421	131	481	401	151	501	141
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	M	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	M
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
INTEGUMENTARY SYSTEM																									
Mammary gland	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma										X								X							
Fibroadenoma, two																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue, fibrosarcoma																		X							
Subcutaneous tissue, sarcoma							X																		
Vulva, papilloma																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma benign																									
Cerebellum, meningioma malignant		X																							
Spinal cord	+																								
RESPIRATORY SYSTEM																									
Larynx	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Eye	+											+	+								+				
Lacrimal gland												+													
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X	X	X	X		X					X				X	X	X	X	X	X	X	

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: CHAMBER CONTROL (Continued)

DAYS ON STUDY	727	727	727	727	727	727	727	727	727	727	727	728	728	728	728	728	729	729	729	729	729	729	729	729	729	TOTAL:
CARCASS ID	031	041	061	081	091	101	371	391	411	441	471	111	181	191	211	221	231	241	251	271	291	301	311	321	331	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibroadenoma		X		X								X				X						X				7
Fibroadenoma, two								X																		1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma															X											1
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, sarcoma																										1
Vulva, papilloma											X															1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma benign																	X									1
Cerebellum, meningioma malignant																										1
Spinal cord																										1
RESPIRATORY SYSTEM																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSES SYSTEM																										
Eye																										4
Lacrimal gland											+															2
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SYSTEMIC LESIONS																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X		X													X			X			X		18

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: 2 ppm

DAYS ON STUDY	428	545	559	563	577	584	587	603	612	619	639	681	689	703	706	711	727	727	727	727	727	728	728	728	728
CARCASS ID	23551	22041	20511	21161	22291	20111	21131	23001	24011	20811	22251	21191	23811	23411	21101	20221	20031	20041	20061	20071	20091	21111	21121	21141	21151
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	M	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery			+	+			+			+											+				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	M	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Bilateral, pheochromocytoma benign				X																					
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X	X	X		X	X			X	X	X	X	X				X		X	X	X		X
Pars distalis, adenoma, two																	X								
Pars distalis, carcinoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma				X								X											X		
C-cell, carcinoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+
Adenoma			X																						
Carcinoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deciduoma benign																								X	
Polyp stromal	X														X						X		X	X	
Polyp stromal, two																									
Sarcoma stromal						X																			
Bilateral, polyp stromal																						X			
Vagina									+																
Schwannoma malignant, metastatic, urinary bladder									X																

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 2 ppm (Continued)

DAYS ON STUDY	728	728	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	TOTAL:
CARCASS ID	21771	21881	22001	22111	22221	22331	22661	22771	22881	23111	23211	23311	23361	23711	23911	24111	24221	24331	24441	24551	24661	24771	24881	24991	25001	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																						X				1
Mesentery				+																						6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue																+										1
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma benign											X	X			X											3
Bilateral, pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma		X	X	X					X			X	X	X			X	X		X	X			X	27
Pars distalis, adenoma, two										X																2
Pars distalis, carcinoma							X								X											2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma				X										X										X		6
C-cell, carcinoma							X															X				2
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma								X		X							X									4
Carcinoma												X														1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Deciduoma benign																										1
Polyp stromal								X		X					X											8
Polyp stromal, two																				X						1
Sarcoma stromal																										1
Bilateral, polyp stromal																	X						X			3
Vagina																										1
Schwannoma malignant, metastatic, urinary bladder																										1

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 2 ppm
(Continued)

DAYS ON STUDY	428	545	559	563	577	584	587	603	612	619	639	681	689	703	706	716	727	727	727	727	727	728	728	728	728
CARCASS ID	23511	22411	20511	21611	22911	20111	21311	23011	24011	20811	22511	21911	23811	23411	21011	20211	20311	20411	20611	20711	20911	21111	21211	21411	21511
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Fibroadenoma	X									X			X	X			X	X			X				
Fibroadenoma, two																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Basosquamous tumor malignant														X											
Papilloma squamous				X																					
Subcutaneous tissue, fibroma																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Larynx	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																		X						X	
Alveolar/bronchiolar carcinoma															X				X						X
Alveolar/bronchiolar carcinoma, multiple														X											
Alveolar/bronchiolar carcinoma, two																				X	X	X			
Chordoma, metastatic, uncertain primary site																									
Squamous cell carcinoma											X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Eye				+				+															+		
Zymbal gland					+																				
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant									X																
Transitional epithelium, papilloma																									
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X								X						X		X				X			

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 2 ppm (Continued)

DAYS ON STUDY	728	728	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	TOTAL:
CARCASS ID	21771	21811	22201	22211	22221	22231	22261	22271	22281	23111	23211	23311	23611	23711	23911	24111	24211	24311	24411	24511	24611	24711	24811	24911	25011	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	46
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma				X																						1
Fibroadenoma	X	X			X													X								11
Fibroadenoma, two																	X									1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																								X		1
Basosquamous tumor malignant																										1
Papilloma squamous													X													2
Subcutaneous tissue, fibroma																					X					1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	50
RESPIRATORY SYSTEM																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	49
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X														X	X						X	6
Alveolar/bronchiolar carcinoma		X		X							X	X				X		X		X					X	11
Alveolar/bronchiolar carcinoma, multiple																										1
Alveolar/bronchiolar carcinoma, two					X		X							X	X											7
Chordoma, metastatic, uncertain primary site											X															1
Squamous cell carcinoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Eye																										3
Zymbal gland																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																										1
Transitional epithelium, papilloma																				X						1
SYSTEMIC LESIONS																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X						X						X					X		X					10

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: 5 ppm

DAYS ON STUDY	356	475	495	512	520	534	545	560	567	570	576	584	590	616	626	633	646	658	665	667	670	688	689	695	695
CARCASS ID	11991	14711	12511	10811	11411	14411	11611	14111	13511	10711	12411	14211	11011	10111	11811	12211	12811	10611	14911	13111	10311	12311	12711	13011	14611
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Intestine large, colon	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Intestine large, rectum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Leiomyoma																									
Intestine small, ileum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	
Intestine small, jejunum	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	A	+	+	+	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																								X	
Hepatocellular carcinoma																									
Sarcoma, metastatic, lung																X									
Mesentery						+						+					+			+	+				
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Leukemia, multifocal																									
Sarcoma, metastatic, uncertain primary site																					X				
Sarcoma, metastatic, uterus																	X								
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Alveolar/bronchiolar carcinoma, metastatic, lung														X				X							
Mixed tumor malignant, metastatic, lung																X									
Sarcoma, metastatic, lung																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X										X						X					
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung																									
Sarcoma, metastatic, multiple, lung																									
Epicardium, carcinoma, metastatic, lung																									X
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma														X											
Alveolar/bronchiolar carcinoma, metastatic, lung																						X		X	
Carcinoma																									X
Carcinoma, metastatic, lung																									
Medulla, alveolar/bronchiolar carcinoma, metastatic, lung																		X							
Adrenal gland, medulla	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M		+	+	+	+	+	+	+
Pheochromocytoma complex																									
Pheochromocytoma benign																									
Sarcoma, metastatic, lung																									
Bilateral, pheochromocytoma benign	X																								
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Carcinoma												X													
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X	X			X	X				X		X				X		X	X			
Pars distalis, adenoma, two																									
Pars distalis, alveolar/bronchiolar carcinoma, metastatic, lung																		X							
Pars intermedia, adenoma											X														
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
C-cell, carcinoma																							X		
Follicular cell, adenoma																									
Follicular cell, carcinoma															X										

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 5 ppm (Continued)

DAYS ON STUDY	6 9 6	6 9 6	7 0 0	7 0 2	7 1 4	7 1 7	7 1 9	7 2 0	7 2 1	7 2 2	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	TOTAL:
CARCASS ID	1 0 5 1	1 4 0 1	1 2 1 1	1 0 4 1	1 0 2 1	1 1 2 1	1 3 3 1	1 3 4 1	1 0 9 1	1 2 6 1	1 1 1 1	1 1 3 1	1 1 5 1	1 1 7 1	1 2 0 1	1 2 9 1	1 3 2 1	1 3 6 1	1 3 7 1	1 3 8 1	1 3 9 1	1 4 3 1	1 4 5 1	1 4 8 1	1 5 0 1	TISSUES TUMORS
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyoma																								X	1
Intestine small, ileum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																									1
Hepatocellular carcinoma	X																								1
Sarcoma, metastatic, lung																									1
Mesentery									+	+						+									8
Alveolar/bronchiolar carcinoma, metastatic, lung										X															1
Leukemia, multifocal									X																1
Sarcoma, metastatic, uncertain primary site																									1
Sarcoma, metastatic, uterus																									1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung																				X					3
Mixed tumor malignant, metastatic, lung																									1
Sarcoma, metastatic, lung							X																		1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung		X																							1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																									3
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung		X																							1
Sarcoma, metastatic, multiple, lung							X																		1
Epicardium, carcinoma, metastatic, lung																									1
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung		X																							1
Adrenal gland, cortex	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma						X																X			3
Alveolar/bronchiolar carcinoma, metastatic, lung	X													X											4
Carcinoma				X																					1
Carcinoma, metastatic, lung																									1
Medulla, alveolar/bronchiolar carcinoma, metastatic, lung										X															2
Adrenal gland, medulla	+	+	+	+	+	+	+	+	M		+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pheochromocytoma complex																			X						1
Pheochromocytoma benign											X												X		2
Sarcoma, metastatic, lung							X																		1
Bilateral, pheochromocytoma benign																									1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																									1
Parathyroid gland	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+		+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X					X		X		X	X		X	X	X		X	X	X	X			X	X	24
Pars distalis, adenoma, two																	X								1
Pars distalis, alveolar/bronchiolar carcinoma, metastatic, lung																									1
Pars intermedia, adenoma																									1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma	X													X	X			X							4
C-cell, carcinoma																									1
Follicular cell, adenoma																		X							1
Follicular cell, carcinoma																									1

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 5 ppm (Continued)

DAYS ON STUDY	356	475	495	512	520	534	545	560	567	570	576	584	590	616	626	633	646	658	665	667	670	688	689	695	695
CARCASS ID	11911	14711	12511	10811	11411	14411	11611	14111	13511	10711	12411	14211	11011	10111	11811	12211	12281	10611	14911	13111	10311	12311	12711	13011	14611
GENERAL BODY SYSTEM																									
Tissue, NOS																					+				
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung																									
Sarcoma, metastatic, uncertain primary site																					X				
Squamous cell carcinoma, metastatic, lung																									
GENITAL SYSTEM																									
Clitoral gland	+	+	+	+	+		+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	M	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																X		X		X		X			
Bilateral, carcinoma, metastatic, lung																									X
Bilateral, sarcoma, metastatic, lung																X									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal				X																					
Sarcoma			X														X								
HEMATOPOIETIC SYSTEM																									
Blood																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, lung																X									
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung													X					X				X			
Mediastinal, carcinoma, metastatic, lung																									X
Serosa, mediastinal, sarcoma, metastatic, uncertain primary site																					X				
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, lung																								X	
Thymus	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	M	+	M
Alveolar/bronchiolar carcinoma, metastatic, lung														X		X				X					
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Fibroadenoma						X																	X		
Fibroadenoma, two															X										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Papilloma squamous										X															
Subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic																									
Subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic, lung																								X	
Subcutaneous tissue, fibroma																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant									X						X										
Spinal cord												+													

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 5 ppm (Continued)

DAYS ON STUDY	6 9 6	6 9 6	7 0 0	7 0 2	7 1 4	7 1 7	7 1 9	7 2 0	7 2 1	7 2 2	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	TOTAL:
CARCASS ID	1 0 5 1	1 4 0 1	1 2 1 1	1 0 4 1	1 0 2 1	1 1 2 1	1 3 3 1	1 3 4 1	1 0 9 1	1 2 6 1	1 1 1 1	1 1 3 1	1 1 5 1	1 1 7 1	1 2 0 1	1 2 9 1	1 3 2 1	1 3 6 1	1 3 7 1	1 3 8 1	1 3 9 1	1 4 3 1	1 4 5 1	1 4 8 1	1 5 0 1	TISSUES TUMORS
GENERAL BODY SYSTEM																											
Tissue, NOS		+	+																								3
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung		X																									1
Sarcoma, metastatic, uncertain primary site																											1
Squamous cell carcinoma, metastatic, lung			X																								1
GENITAL SYSTEM																											
Clitoral gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+		44
Ovary	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+		48
Alveolar/bronchiolar carcinoma, metastatic, lung		X																									5
Bilateral, carcinoma, metastatic, lung																											1
Bilateral, sarcoma, metastatic, lung																											1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Polyp stromal					X							X						X	X								5
Sarcoma																											2
HEMATOPOIETIC SYSTEM																											
Blood									I																		
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Sarcoma, metastatic, lung																											1
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung		X		X																							5
Mediastinal, carcinoma, metastatic, lung																											1
Serosa, mediastinal, sarcoma, metastatic, uncertain primary site																											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+		49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Squamous cell carcinoma, metastatic, lung																											1
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+		42
Alveolar/bronchiolar carcinoma, metastatic, lung																		X									4
INTEGUMENTARY SYSTEM																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Adenoma																								X			1
Fibroadenoma				X																					X		4
Fibroadenoma, two									X																		2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Keratoacanthoma																								X			1
Papilloma squamous																											1
Subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic										X																	1
Subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic, lung																											1
Subcutaneous tissue, fibroma																			X								1
MUSCULOSKELETAL SYSTEM																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		49
NERVOUS SYSTEM																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Astrocytoma malignant																											2
Spinal cord																											1

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 5 ppm (Continued)

DAYS ON STUDY	356	475	495	512	520	534	545	560	567	570	576	584	590	616	626	633	646	658	665	667	670	688	689	695	695
CARCASS ID	11911	14711	12511	10811	11411	14411	11611	14111	13511	10711	12411	14211	11011	10111	11811	12211	12281	10611	14911	13111	10311	12311	12711	13011	14611
RESPIRATORY SYSTEM																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X					X											
Alveolar/bronchiolar carcinoma		X	X							X															
Alveolar/bronchiolar carcinoma, multiple	X			X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X	X	X	X
Alveolar/bronchiolar carcinoma, two																					X				
Mixed tumor malignant, multiple																X									
Osteosarcoma, metastatic, uncertain primary site																		X							
Sarcoma, multiple																									
Squamous cell carcinoma				X							X										X				
Squamous cell carcinoma, multiple																								X	
Mediastinum, carcinoma, metastatic, lung																									X
Mediastinum, squamous cell carcinoma, metastatic, lung																									
Pleura, mediastinum, sarcoma, metastatic, uncertain primary site																					X				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
None																									
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung													X	X		X				X		X		X	
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung																		X							
Carcinoma, metastatic, lung																									X
Osteosarcoma, metastatic, uncertain primary site																		X							
Sarcoma, metastatic, lung																X									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia																									
Leukemia mononuclear		X					X									X									

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: 5 ppm (Continued)

DAYS ON STUDY	6 9 6	6 9 6	7 0 0	7 0 2	7 1 4	7 1 7	7 1 9	7 2 0	7 2 1	7 2 2	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	7 2 8	TOTAL:
CARCASS ID	1 0 5 1	1 4 0 1	1 2 1 1	1 0 4 1	1 0 2 1	1 1 2 1	1 3 3 1	1 3 4 1	1 0 9 1	1 2 6 1	1 1 1 1	1 1 3 1	1 1 5 1	1 1 7 1	1 2 0 1	1 2 9 1	1 3 2 1	1 3 6 1	1 3 7 1	1 3 8 1	1 3 9 1	1 4 3 1	1 4 5 1	1 4 8 1	1 5 0 1	TISSUES TUMORS
RESPIRATORY SYSTEM																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma								X																		3
Alveolar/bronchiolar carcinoma																										3
Alveolar/bronchiolar carcinoma, multiple	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	44
Alveolar/bronchiolar carcinoma, two						X															X					3
Mixed tumor malignant, multiple																										1
Osteosarcoma, metastatic, uncertain primary site																										1
Sarcoma, multiple							X																			1
Squamous cell carcinoma	X			X						X			X						X	X		X				10
Squamous cell carcinoma, multiple			X																							2
Mediastinum, carcinoma, metast., lung																										1
Mediastinum, squamous cell carcinoma, metastatic, lung			X																							1
Pleura, mediastinum, sarcoma, metastatic, uncertain primary site																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
None																										
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung		X																								7
Alveolar/bronchiolar carcinoma, metastatic, multiple, lung										X																2
Carcinoma, metastatic, lung																										1
Osteosarcoma, metastatic, uncertain primary site																										1
Sarcoma, metastatic, lung							X																			2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SYSTEMIC LESIONS																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia									X																	1
Leukemia mononuclear								X										X		X						6

TABLE B3. ANALYSIS OF PRIMARY NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	2 ppm	5 ppm
Adrenal Cortex: Adenoma			
Overall Rates (a)	0/50 (0%)	0/49 (0%)	3/49 (6%)
Adjusted Rates (b)	0.0%	0.0%	13.7%
Terminal Rates (c)	0/25 (0%)	0/34 (0%)	1/15 (7%)
Day of First Observation			616
Life Table Tests (d)	P=0.015	(e)	P=0.082
Logistic Regression Tests (d)	P=0.029	(e)	P=0.115
Cochran-Armitage Trend Test (d)	P=0.032		
Fisher Exact Test (d)		(e)	P=0.117
Adrenal Cortex: Adenoma or Carcinoma			
Overall Rates (a)	0/50 (0%)	0/49 (0%)	4/49 (8%)
Adjusted Rates (b)	0.0%	0.0%	17.7%
Terminal Rates (c)	0/25 (0%)	0/34 (0%)	1/15 (7%)
Day of First Observation			616
Life Table Tests (d)	P=0.004	(e)	P=0.040
Logistic Regression Tests (d)	P=0.010	(e)	P=0.058
Cochran-Armitage Trend Test (d)			
Fisher Exact Test (d)	P=0.011	(e)	P=0.056
Adrenal Medulla: Pheochromocytoma			
Overall Rates (a)	2/43 (5%)	4/48 (8%)	3/47 (6%)
Adjusted Rates (b)	9.1%	10.8%	15.1%
Terminal Rates (c)	2/22 (9%)	3/34 (9%)	2/15 (13%)
Day of First Observation	727	563	356
Life Table Tests (d)	P=0.306	P=0.524	P=0.365
Logistic Regression Tests (d)	P=0.507	P=0.447	P=0.540
Cochran-Armitage Trend Test (d)	P=0.506		
Fisher Exact Test (d)		P=0.392	P=0.543
Adrenal Medulla: Pheochromocytoma or Complex Pheochromocytoma			
Overall Rates (a)	2/43 (5%)	4/48 (8%)	4/47 (9%)
Adjusted Rates (b)	9.1%	10.8%	21.6%
Terminal Rates (c)	2/22 (9%)	3/34 (9%)	3/15 (20%)
Day of First Observation	727	563	356
Life Table Tests (d)	P=0.153	P=0.524	P=0.201
Logistic Regression Tests (d)	P=0.341	P=0.447	P=0.378
Cochran-Armitage Trend Test (d)	P=0.343		
Fisher Exact Test (d)		P=0.392	P=0.382
Clitoral Gland: Adenoma			
Overall Rates (a)	1/47 (2%)	4/48 (8%)	0/44 (0%)
Adjusted Rates (b)	4.0%	11.0%	0.0%
Terminal Rates (c)	1/25 (4%)	3/33 (9%)	0/14 (0%)
Day of First Observation	727	559	
Life Table Tests (d)	P=0.459N	P=0.264	P=0.616N
Logistic Regression Tests (d)	P=0.349N	P=0.204	P=0.616N
Cochran-Armitage Trend Test (d)	P=0.338N		
Fisher Exact Test (d)		P=0.187	P=0.516N
Clitoral Gland: Adenoma or Carcinoma			
Overall Rates (a)	3/47 (6%)	5/48 (10%)	0/44 (0%)
Adjusted Rates (b)	12.0%	14.0%	0.0%
Terminal Rates (c)	3/25 (12%)	4/33 (12%)	0/14 (0%)
Day of First Observation	727	559	
Life Table Tests (d)	P=0.203N	P=0.507	P=0.238N
Logistic Regression Tests (d)	P=0.125N	P=0.434	P=0.238N
Cochran-Armitage Trend Test (d)	P=0.115N		
Fisher Exact Test (d)		P=0.369	P=0.133N

TABLE B3. ANALYSIS OF PRIMARY NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	0/50 (0%)	6/50 (12%)	3/50 (6%)
Adjusted Rates (b)	0.0%	17.6%	10.3%
Terminal Rates (c)	0/25 (0%)	6/34 (18%)	0/15 (0%)
Day of First Observation		727	567
Life Table Tests (d)	P=0.091	P=0.039	P=0.104
Logistic Regression Tests (d)	P=0.208	P=0.039	P=0.116
Cochran-Armitage Trend Test (d)	P=0.226		
Fisher Exact Test (d)		P=0.013	P=0.121
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	0/50 (0%)	19/50 (38%)	50/50 (100%)
Adjusted Rates (b)	0.0%	52.7%	100.0%
Terminal Rates (c)	0/25 (0%)	17/34 (50%)	15/15 (100%)
Day of First Observation		703	356
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Logistic Regression Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P<0.001	P<0.001
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	0/50 (0%)	22/50 (44%)	50/50 (100%)
Adjusted Rates (b)	0.0%	61.0%	100.0%
Terminal Rates (c)	0/25 (0%)	20/34 (59%)	15/15 (100%)
Day of First Observation		703	356
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Logistic Regression Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P<0.001	P<0.001
Lung: Squamous Cell Carcinoma			
Overall Rates (a)	0/50 (0%)	1/50 (2%)	12/50 (24%)
Adjusted Rates (b)	0.0%	2.5%	46.5%
Terminal Rates (c)	0/25 (0%)	0/34 (0%)	4/15 (27%)
Day of First Observation		639	512
Life Table Tests (d)	P<0.001	P=0.527	P<0.001
Logistic Regression Tests (d)	P<0.001	P=0.478	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.500	P<0.001
Mammary Gland: Fibroadenoma			
Overall Rates (f)	8/50 (16%)	12/50 (24%)	6/50 (12%)
Adjusted Rates (b)	28.1%	30.7%	23.1%
Terminal Rates (c)	6/25 (24%)	8/34 (24%)	1/15 (7%)
Day of First Observation	590	428	534
Life Table Tests (d)	P=0.558	P=0.467	P=0.607
Logistic Regression Tests (d)	P=0.298N	P=0.303	P=0.390N
Cochran-Armitage Trend Test (d)	P=0.294N		
Fisher Exact Test (d)		P=0.227	P=0.387N
Mammary Gland: Adenoma or Fibroadenoma			
Overall Rates (f)	8/50 (16%)	12/50 (24%)	7/50 (14%)
Adjusted Rates (b)	28.1%	30.7%	28.6%
Terminal Rates (c)	6/25 (24%)	8/34 (24%)	2/15 (13%)
Day of First Observation	590	428	534
Life Table Tests (d)	P=0.423	P=0.467	P=0.468
Logistic Regression Tests (d)	P=0.407N	P=0.303	P=0.507N
Cochran-Armitage Trend Test (d)	P=0.398N		
Fisher Exact Test (d)		P=0.227	P=0.500N

TABLE B3. ANALYSIS OF PRIMARY NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
Mammary Gland: Adenoma, Fibroadenoma, or Adenocarcinoma			
Overall Rates (f)	8/50 (16%)	13/50 (26%)	7/50 (14%)
Adjusted Rates (b)	28.1%	33.4%	28.6%
Terminal Rates (c)	6/25 (24%)	9/34 (26%)	2/15 (13%)
Day of First Observation	590	428	534
Life Table Tests (d)	P=0.421	P=0.387	P=0.468
Logistic Regression Tests (d)	P=0.397N	P=0.234	P=0.507N
Cochran-Armitage Trend Test (d)	P=0.386N		
Fisher Exact Test (d)		P=0.163	P=0.500N
Pituitary Gland/Pars Distalis: Adenoma			
Overall Rates (a)	28/50 (56%)	29/48 (60%)	25/50 (50%)
Adjusted Rates (b)	79.4%	66.8%	87.2%
Terminal Rates (c)	18/25 (72%)	19/33 (58%)	12/15 (80%)
Day of First Observation	525	559	512
Life Table Tests (d)	P=0.188	P=0.189N	P=0.226
Logistic Regression Tests (d)	P=0.289N	P=0.522N	P=0.333N
Cochran-Armitage Trend Test (d)	P=0.282N		
Fisher Exact Test (d)		P=0.406	P=0.344N
Pituitary Gland/Pars Distalis: Adenoma or Carcinoma			
Overall Rates (a)	29/50 (58%)	31/48 (65%)	25/50 (50%)
Adjusted Rates (b)	80.0%	71.5%	87.2%
Terminal Rates (c)	18/25 (72%)	21/33 (64%)	12/15 (80%)
Day of First Observation	525	559	512
Life Table Tests (d)	P=0.236	P=0.223N	P=0.288
Logistic Regression Tests (d)	P=0.208N	P=0.575	P=0.257N
Cochran-Armitage Trend Test (d)	P=0.206N		
Fisher Exact Test (d)		P=0.323	P=0.274N
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	3/49 (6%)	6/50 (12%)	4/50 (8%)
Adjusted Rates (b)	11.3%	15.9%	23.2%
Terminal Rates (c)	2/25 (8%)	4/34 (12%)	3/15 (20%)
Day of First Observation	674	563	696
Life Table Tests (d)	P=0.274	P=0.408	P=0.308
Logistic Regression Tests (d)	P=0.457	P=0.301	P=0.451
Cochran-Armitage Trend Test (d)	P=0.502		
Fisher Exact Test (d)		P=0.254	P=0.511
Thyroid Gland: C-Cell Carcinoma			
Overall Rates (a)	3/49 (6%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	12.0%	5.9%	3.6%
Terminal Rates (c)	3/25 (12%)	2/34 (6%)	0/15 (0%)
Day of First Observation	727	727	689
Life Table Tests (d)	P=0.366N	P=0.360N	P=0.444N
Logistic Regression Tests (d)	P=0.273N	P=0.360N	P=0.342N
Cochran-Armitage Trend Test (d)	P=0.233N		
Fisher Exact Test (d)		P=0.490N	P=0.301N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	6/49 (12%)	8/50 (16%)	5/50 (10%)
Adjusted Rates (b)	22.9%	21.5%	25.9%
Terminal Rates (c)	5/25 (20%)	6/34 (18%)	3/15 (20%)
Day of First Observation	674	563	689
Life Table Tests (d)	P=0.454	P=0.604N	P=0.502
Logistic Regression Tests (d)	P=0.464N	P=0.522	P=0.566N
Cochran-Armitage Trend Test (d)	P=0.399N		
Fisher Exact Test (d)		P=0.403	P=0.486N

TABLE B3. ANALYSIS OF PRIMARY NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
Uterus: Stromal Polyp			
Overall Rates (f)	11/50 (22%)	12/50 (24%)	5/50 (10%)
Adjusted Rates (b)	31.0%	32.7%	25.4%
Terminal Rates (c)	5/25 (20%)	10/34 (29%)	3/15 (20%)
Day of First Observation	454	428	512
Life Table Tests (d)	P=0.212N	P=0.428N	P=0.214N
Logistic Regression Tests (d)	P=0.064N	P=0.488	P=0.088N
Cochran-Armitage Trend Test (d)	P=0.065N		
Fisher Exact Test (d)		P=0.500	P=0.086N
Hematopoietic System: Mononuclear Leukemia or Leukemia			
Overall Rates (f)	18/50 (36%)	10/50 (20%)	7/50 (14%)
Adjusted Rates (b)	45.2%	26.3%	28.4%
Terminal Rates (c)	5/25 (20%)	7/34 (21%)	2/15 (13%)
Day of First Observation	491	545	475
Life Table Tests (d)	P=0.044N	P=0.021N	P=0.049N
Logistic Regression Tests (d)	P=0.009N	P=0.067N	P=0.011N
Cochran-Armitage Trend Test (d)	P=0.009N		
Fisher Exact Test (d)		P=0.059N	P=0.010N
All Sites: Benign Tumors			
Overall Rates (f)	37/50 (74%)	42/50 (84%)	37/50 (74%)
Adjusted Rates (b)	92.2%	91.2%	97.0%
Terminal Rates (c)	22/25 (88%)	30/34 (88%)	14/15 (93%)
Day of First Observation	454	428	356
Life Table Tests (d)	P=0.038	P=0.232N	P=0.079
Logistic Regression Tests (d)	P=0.462N	P=0.365	P=0.541N
Cochran-Armitage Trend Test (d)	P=0.494N		
Fisher Exact Test (d)		P=0.163	P=0.590N
All Sites: Malignant Tumors			
Overall Rates (f)	24/50 (48%)	31/50 (62%)	50/50 (100%)
Adjusted Rates (b)	58.3%	73.6%	100.0%
Terminal Rates (c)	9/25 (36%)	23/34 (68%)	15/15 (100%)
Day of First Observation	251	545	356
Life Table Tests (d)	P<0.001	P=0.557	P<0.001
Logistic Regression Tests (d)	P<0.001	P=0.139	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.114	P<0.001
All Sites: All Tumors			
Overall Rates (f)	47/50 (94%)	49/50 (98%)	50/50 (100%)
Adjusted Rates (b)	97.9%	98.0%	100.0%
Terminal Rates (c)	24/25 (96%)	33/34 (97%)	15/15 (100%)
Day of First Observation	251	428	356
Life Table Tests (d)	P=0.013	P=0.084N	P=0.046
Logistic Regression Tests (d)	P=0.119	P=0.498	P=0.187
Cochran-Armitage Trend Test (d)	P=0.075		
Fisher Exact Test (d)		P=0.309	P=0.121

(a) Number of tumor-bearing animals/number of animals examined microscopically at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or a lower incidence in a dosed group than in controls is indicated by (N).
(e) No P value is reported because no tumors were observed in the 2-ppm and control groups
(f) Number of tumor-bearing animals/number of animals examined grossly at the site

TABLE B4a. HISTORICAL INCIDENCE OF ALVEOLAR/BRONCHIOLAR NEOPLASMS IN FEMALE F344/N RATS (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence for Chamber Controls in NTP Studies (b)			
Propylene oxide	0/48	0/48	0/48
Methyl methacrylate	0/50	0/50	0/50
Propylene	0/49	0/49	0/49
1,2-Epoxybutane	1/50	1/50	2/50
Dichloromethane	1/50	0/50	1/50
Tetrachloroethylene	0/50	1/50	1/50
Bromoethane	0/50	0/50	0/50
TOTAL	2/347 (0.6%)	2/347 (0.6%)	4/347 (1.2%)
SD (c)	0.98%	0.98%	1.57%
Range (d)			
High	1/50	1/50	2/50
Low	0/50	0/50	0/50
Overall Historical Incidence for Untreated Controls in NTP Studies			
TOTAL	20/1,639 (1.2%)	5/1,639 (0.3%)	25/1,639 (1.5%)
SD (c)	1.58%	0.73%	1.59%
Range (d)			
High	3/50	1/50	3/50
Low	0/50	0/50	0/50

(a) Data as of March 1, 1989, for studies of at least 104 weeks
(b) All inhalation studies included in the NTP historical data base were conducted at Battelle Pacific Northwest Laboratories.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE B4b. HISTORICAL INCIDENCE OF SQUAMOUS CELL LUNG NEOPLASMS IN FEMALE F344/N RATS (a)

Historical incidence for chamber controls in NTP studies: 0/347

Overall historical incidence for untreated controls in NTP studies: 0/1,639

(a) Data as of March 1, 1989, for studies of at least 104 weeks

TABLE B4c. HISTORICAL INCIDENCE OF ADRENAL CORTICAL NEOPLASMS IN FEMALE F344/N RATS (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence for Chamber Controls in NTP Studies (b)			
Propylene oxide	1/48	0/48	1/48
Methyl methacrylate	0/49	0/49	0/49
Propylene	1/47	0/47	1/47
1,2-Epoxybutane	1/50	0/50	1/50
Dichloromethane	0/50	0/50	0/50
Tetrachloroethylene	2/50	0/50	2/50
Bromoethane	1/50	0/50	1/50
TOTAL	6/344 (1.7%)	0/344 (0.0%)	6/344 (1.7%)
SD (c)	1.39%	0.00%	1.39%
Range (d)			
High	2/50	0/50	2/50
Low	0/50	0/50	0/50
Overall Historical Incidence for Untreated Controls in NTP Studies			
TOTAL	(e) 48/1,634 (2.9%)	5/1,634 (0.3%)	(e) 53/1,634 (3.2%)
SD (c)	2.97%	0.73%	3.05%
Range (d)			
High	6/50	1/49	6/50
Low	0/50	0/50	0/50

(a) Data as of March 1, 1989, for studies of at least 104 weeks
(b) All inhalation studies included in the NTP historical data base were conducted at Battelle Pacific Northwest Laboratories.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Includes four adenomas, NOS

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	2 ppm	5 ppm
DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Early deaths			
Natural death	6	3	8
Moribund sacrifice	19	13	27
Survivors			
Terminal sacrifice	25	34	15
Animals examined microscopically	50	50	50
ALIMENTARY SYSTEM			
Intestine large, cecum	(49)	(48)	(48)
Inflammation, chronic active			1 (2%)
Inflammation, necrotizing, acute	1 (2%)		
Parasite metazoan	3 (6%)	2 (4%)	
Intestine large, colon	(50)	(49)	(48)
Parasite metazoan	5 (10%)	10 (20%)	5 (10%)
Artery, inflammation, chronic active, focal	1 (2%)		
Muscularis, mineralization, multifocal		1 (2%)	
Intestine large, rectum	(49)	(49)	(49)
Parasite metazoan	7 (14%)	7 (14%)	6 (12%)
Muscularis, mineralization, multifocal		1 (2%)	
Intestine small, ileum	(46)	(49)	(46)
Infiltration cellular, lymphocytic, diffuse			1 (2%)
Inflammation, chronic active	1 (2%)		
Liver	(50)	(50)	(50)
Angiectasis, focal	3 (6%)	2 (4%)	
Angiectasis, multifocal			1 (2%)
Basophilic focus	2 (4%)	2 (4%)	8 (16%)
Basophilic focus, multiple	24 (48%)	29 (58%)	15 (30%)
Basophilic focus, two	1 (2%)	6 (12%)	7 (14%)
Cytomegaly, multifocal		1 (2%)	
Cytoplasmic alteration, focal	1 (2%)	2 (4%)	
Eosinophilic focus	1 (2%)	2 (4%)	
Fatty change, diffuse	6 (12%)	2 (4%)	
Fatty change, focal	2 (4%)		
Fatty change, multifocal	3 (6%)	6 (12%)	6 (12%)
Fibrosis, multifocal		1 (2%)	1 (2%)
Granuloma, multifocal	15 (30%)	28 (56%)	16 (32%)
Hemorrhage, acute, multifocal			2 (4%)
Hepatodiaphragmatic nodule	5 (10%)	4 (8%)	5 (10%)
Hyperplasia, nodular		1 (2%)	1 (2%)
Inflammation, chronic active, multifocal	1 (2%)		
Mitotic alteration	2 (4%)	3 (6%)	1 (2%)
Mixed cell focus	2 (4%)	2 (4%)	1 (2%)
Mixed cell focus, multiple	1 (2%)		
Necrosis, acute, focal	1 (2%)		1 (2%)
Necrosis, acute, multifocal	2 (4%)	2 (4%)	2 (4%)
Necrosis, subacute, focal	1 (2%)		
Vacuolization cytoplasmic, multifocal	1 (2%)	2 (4%)	
Bile duct, hyperplasia, multifocal	10 (20%)	6 (12%)	3 (6%)
Centrilobular, atrophy, diffuse	2 (4%)		1 (2%)
Centrilobular, atrophy, multifocal	3 (6%)	2 (4%)	2 (4%)
Centrilobular, congestion, multifocal		1 (2%)	
Centrilobular, fatty change, diffuse	3 (6%)		2 (4%)
Centrilobular, necrosis, acute, diffuse			1 (2%)
Centrilobular, necrosis, acute, multifocal		1 (2%)	1 (2%)
Median lobe, hepatodiaphragmatic nodule			1 (2%)
Periportal, cytomegaly, diffuse		1 (2%)	
Periportal, fatty change, diffuse	1 (2%)	1 (2%)	1 (2%)
Periportal, fatty change, multifocal	2 (4%)	2 (4%)	2 (4%)
Periportal, inflammation, chronic, multifocal	1 (2%)		

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
ALIMENTARY SYSTEM			
Liver (Continued)	(50)	(50)	(50)
Portal, inflammation, chronic, multifocal	1 (2%)	1 (2%)	
Serosa, inflammation, proliferative, multifocal			1 (2%)
Mesentery	(4)	(6)	(8)
Accessory spleen	2 (50%)		
Artery, mineralization, multifocal		1 (17%)	
Fat, necrosis, focal	2 (50%)	3 (50%)	3 (38%)
Fat, necrosis, multifocal		1 (17%)	1 (13%)
Vein, thrombus		1 (17%)	
Pancreas	(50)	(50)	(49)
Ectopic tissue			1 (2%)
Inflammation, chronic, multifocal	1 (2%)	1 (2%)	
Necrosis, acute, focal			1 (2%)
Acinus, atrophy			2 (4%)
Acinus, atrophy, diffuse	1 (2%)		2 (4%)
Acinus, atrophy, focal	1 (2%)		1 (2%)
Acinus, atrophy, multifocal	12 (24%)	8 (16%)	5 (10%)
Acinus, hyperplasia, focal	1 (2%)	2 (4%)	
Artery, angiectasis, multifocal		1 (2%)	
Artery, inflammation, chronic active, focal			1 (2%)
Artery, inflammation, chronic active, multifocal	1 (2%)		2 (4%)
Artery, mineralization, multifocal		1 (2%)	
Serosa, inflammation, chronic active		1 (2%)	
Salivary glands	(50)	(50)	(50)
Cytomegaly, focal			1 (2%)
Inflammation, acute, focal			1 (2%)
Acinus, atrophy, focal		1 (2%)	
Duct, hyperplasia, focal	1 (2%)	1 (2%)	
Duct, inflammation, chronic active, focal	1 (2%)		
Stomach, forestomach	(50)	(49)	(50)
Edema			1 (2%)
Erosion, focal	1 (2%)		
Hyperplasia, basal cell, focal			1 (2%)
Inflammation, acute		1 (2%)	1 (2%)
Inflammation, chronic	1 (2%)		
Inflammation, chronic active	6 (12%)	3 (6%)	4 (8%)
Necrosis, acute, focal			1 (2%)
Ulcer, multifocal	1 (2%)		2 (4%)
Ulcer, single	2 (4%)	2 (4%)	1 (2%)
Epithelium, hyperplasia, diffuse	2 (4%)	1 (2%)	2 (4%)
Epithelium, hyperplasia, focal	2 (4%)	1 (2%)	1 (2%)
Epithelium, hyperplasia, multifocal			2 (4%)
Stomach, glandular	(48)	(49)	(50)
Erosion	1 (2%)		
Inflammation, chronic active	1 (2%)	2 (4%)	
Necrosis, subacute, focal		1 (2%)	
Mucosa, mineralization		1 (2%)	
Mucosa, necrosis, acute, focal	1 (2%)		
CARDIOVASCULAR SYSTEM			
Heart	(50)	(50)	(50)
Cardiomyopathy	34 (68%)	39 (78%)	36 (72%)
Mineralization, multifocal		1 (2%)	
Necrosis, acute, multifocal		1 (2%)	1 (2%)
Artery, amyloid deposition, multifocal		1 (2%)	
Artery, adventitia, hyperplasia			1 (2%)
Artery, adventitia, mineralization, focal			1 (2%)
Artery, epicardium, thrombus			1 (2%)
Atrium left, embolus, focal			1 (2%)
Atrium left, inflammation, acute, multifocal			1 (2%)
Atrium left, thrombus	1 (2%)		

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
CARDIOVASCULAR SYSTEM			
Heart (Continued)	(50)	(50)	(50)
Epicardium, inflammation, chronic, focal	1 (2%)		
Mitral valve, degeneration, mucoid			1 (2%)
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(50)	(50)
Capsule, accessory adrenal cortical nodule	2 (4%)	1 (2%)	1 (2%)
Adrenal gland, cortex	(50)	(49)	(49)
Angiectasis, focal		1 (2%)	
Angiectasis, multifocal	1 (2%)	1 (2%)	
Congestion		1 (2%)	2 (4%)
Degeneration, fatty, diffuse	3 (6%)		4 (8%)
Degeneration, fatty, focal	10 (20%)	7 (14%)	9 (18%)
Degeneration, fatty, multifocal	1 (2%)	1 (2%)	7 (14%)
Degeneration, focal			1 (2%)
Hyperplasia, focal	13 (26%)	9 (18%)	4 (8%)
Hyperplasia, multifocal	2 (4%)	5 (10%)	1 (2%)
Hypertrophy, focal	4 (8%)	5 (10%)	2 (4%)
Hypertrophy, multifocal	2 (4%)	1 (2%)	1 (2%)
Adrenal gland, medulla	(43)	(48)	(45)
Fibrosis, diffuse	1 (2%)		
Hyperplasia			1 (2%)
Hyperplasia, focal	1 (2%)	1 (2%)	4 (9%)
Hyperplasia, multifocal	1 (2%)	1 (2%)	3 (7%)
Islets, pancreatic	(50)	(50)	(49)
Atrophy, diffuse			1 (2%)
Hyperplasia, focal	2 (4%)	2 (4%)	
Hyperplasia, multifocal	5 (10%)	6 (12%)	3 (6%)
Parathyroid gland	(44)	(47)	(45)
Hyperplasia, diffuse	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, focal			1 (2%)
Hypertrophy, focal		1 (2%)	
Pituitary gland	(50)	(48)	(50)
Fibrosis, focal	1 (2%)		
Pars distalis, abscess, chronic		1 (2%)	
Pars distalis, angiectasis, focal	1 (2%)	1 (2%)	1 (2%)
Pars distalis, angiectasis, multifocal	4 (8%)	2 (4%)	5 (10%)
Pars distalis, cyst	5 (10%)	2 (4%)	3 (6%)
Pars distalis, cyst, multiple	5 (10%)	3 (6%)	4 (8%)
Pars distalis, hyperplasia		1 (2%)	
Pars distalis, hyperplasia, focal	4 (8%)	4 (8%)	8 (16%)
Pars distalis, hyperplasia, multifocal	1 (2%)		
Pars intermedia, angiectasis, multifocal	1 (2%)	1 (2%)	1 (2%)
Thyroid gland	(49)	(50)	(50)
Cyst	1 (2%)		
Inflammation, granulomatous, focal	1 (2%)		
C-cell, hyperplasia	1 (2%)		
C-cell, hyperplasia, focal	3 (6%)	3 (6%)	2 (4%)
C-cell, hyperplasia, multifocal	11 (22%)	10 (20%)	12 (24%)
Follicular cell, hypertrophy, diffuse	1 (2%)		
GENERAL BODY SYSTEM			
None			

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
GENITAL SYSTEM			
Clitoral gland	(47)	(48)	(44)
Abscess		2 (4%)	1 (2%)
Ectasia	2 (4%)	3 (6%)	6 (14%)
Fibrosis	1 (2%)		
Hyperplasia	1 (2%)		
Hyperplasia, diffuse		1 (2%)	
Hyperplasia, focal	3 (6%)	3 (6%)	1 (2%)
Inflammation, chronic active, focal	1 (2%)		
Inflammation, granulomatous, focal		1 (2%)	
Inflammation, granulomatous, multifocal	2 (4%)		1 (2%)
Necrosis, acute	1 (2%)		
Ovary	(50)	(50)	(48)
Cyst	3 (6%)	5 (10%)	5 (10%)
Bilateral, cyst			1 (2%)
Uterus	(50)	(50)	(50)
Cyst	1 (2%)		3 (6%)
Cyst, multiple	1 (2%)	3 (6%)	
Dilatation	3 (6%)	1 (2%)	4 (8%)
Fibrosis, focal	1 (2%)		
Hyperplasia	1 (2%)	2 (4%)	1 (2%)
Inflammation, suppurative, acute		1 (2%)	
Prolapse	1 (2%)		
Cervix, dilatation	1 (2%)		
Vagina	(2)	(1)	
Inflammation, chronic active	1 (50%)		
Epithelium, hyperplasia, multifocal	1 (50%)		
HEMATOPOIETIC SYSTEM			
Bone marrow	(48)	(50)	(50)
Atrophy	1 (2%)		1 (2%)
Hyperplasia	23 (48%)	20 (40%)	25 (50%)
Myelofibrosis, focal	1 (2%)		
Lymph node	(50)	(50)	(50)
Hyperplasia, plasma cell			1 (2%)
Axillary, hemorrhage, acute		1 (2%)	
Mediastinal, cyst			2 (4%)
Mediastinal, edema		1 (2%)	
Mediastinal, hemorrhage	7 (14%)	7 (14%)	9 (18%)
Mediastinal, hyperplasia			1 (2%)
Mediastinal, hyperplasia, lymphoid			1 (2%)
Mediastinal, inflammation, acute	1 (2%)		
Pancreatic, hemorrhage	4 (8%)	3 (6%)	2 (4%)
Pancreatic, hyperplasia, lymphoid			1 (2%)
Lymph node, mandibular	(49)	(50)	(49)
Hemorrhage, acute	1 (2%)		2 (4%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	2 (4%)
Hyperplasia, plasma cell	2 (4%)	1 (2%)	1 (2%)
Hyperplasia, re cell			1 (2%)
Inflammation, chronic active	1 (2%)		2 (4%)
Lymph node, mesenteric	(47)	(49)	(49)
Atrophy		1 (2%)	
Hemorrhage, acute	6 (13%)	4 (8%)	6 (12%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, re cell	1 (2%)	1 (2%)	5 (10%)
Inflammation, acute			1 (2%)
Inflammation, chronic active	1 (2%)		

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
HEMATOPOIETIC SYSTEM (Continued)			
Spleen	(50)	(50)	(50)
Congestion	2 (4%)	1 (2%)	
Depletion lymphoid	1 (2%)	1 (2%)	1 (2%)
Fibrosis, focal			1 (2%)
Fibrosis, multifocal		1 (2%)	1 (2%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)	14 (28%)
Hyperplasia, re cell			1 (2%)
Pigmentation, hemosiderin	6 (12%)	9 (18%)	8 (16%)
Capsule, fibrosis, multifocal			1 (2%)
Thymus	(48)	(46)	(42)
Congestion			1 (2%)
Depletion lymphoid	1 (2%)		
Epithelial cell, hyperplasia	4 (8%)	1 (2%)	
INTEGUMENTARY SYSTEM			
Mammary gland	(48)	(50)	(50)
Ectasia, diffuse	1 (2%)		4 (8%)
Ectasia, multifocal	16 (33%)	18 (36%)	19 (38%)
Fibrosis, focal			1 (2%)
Galactocele			3 (6%)
Hyperplasia, diffuse	10 (21%)	4 (8%)	5 (10%)
Hyperplasia, focal		2 (4%)	3 (6%)
Hyperplasia, multifocal	3 (6%)	3 (6%)	3 (6%)
Inflammation, granulomatous, multifocal	1 (2%)		1 (2%)
Mineralization, multifocal		1 (2%)	
Duct, ectasia, focal		2 (4%)	
Duct, ectasia, multifocal		1 (2%)	
Duct, hyperplasia, multifocal		1 (2%)	
Skin	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)
Inflammation, chronic active, focal			1 (2%)
Inflammation, subacute, multifocal			1 (2%)
Ulcer			1 (2%)
Epidermis, hyperplasia, focal	1 (2%)		
Subcutaneous tissue, abscess	1 (2%)		
MUSCULOSKELETAL SYSTEM			
Bone	(49)	(50)	(49)
Fibrous osteodystrophy	1 (2%)	1 (2%)	
Osteopetrosis	3 (6%)	4 (8%)	4 (8%)
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
Compression	11 (22%)	12 (24%)	5 (10%)
Hemorrhage, multifocal	2 (4%)		
Hydrocephalus	5 (10%)	5 (10%)	3 (6%)
Choroid plexus, hyperplasia, focal	1 (2%)	1 (2%)	
Spinal cord	(1)		(1)
Degeneration, secondary wallerian, multifocal			1 (100%)
RESPIRATORY SYSTEM			
Larynx	(48)	(49)	(49)
Hyperplasia, papillary, focal	1 (2%)	1 (2%)	1 (2%)
Inflammation, acute		2 (4%)	2 (4%)
Inflammation, chronic	4 (8%)	4 (8%)	6 (12%)
Inflammation, chronic active	7 (15%)	10 (20%)	8 (16%)

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
RESPIRATORY SYSTEM (Continued)			
Lung	(50)	(50)	(50)
Hemorrhage, acute, focal		1 (2%)	
Hemorrhage, acute, multifocal			1 (2%)
Inflammation, necrotizing, acute, multifocal			1 (2%)
Inflammation, necrotizing, subacute, multifocal			1 (2%)
Alveolar epithelium, hyperplasia, atypical, focal		1 (2%)	
Alveolar epithelium, hyperplasia, focal	1 (2%)	5 (10%)	
Alveolar epithelium, hyperplasia, multifocal		38 (76%)	50 (100%)
Alveolus, infiltration cellular, histiocytic, multifocal		1 (2%)	
Artery, mineralization, multifocal	1 (2%)		
Bronchiole, hyperplasia, focal		2 (4%)	
Bronchiole, hyperplasia, multifocal		26 (52%)	48 (96%)
Bronchiole, alveolus, inflammation, suppurative, acute, multifocal			2 (4%)
Bronchus, hyperplasia, papillary, focal		1 (2%)	
Interstitium, inflammation, chronic, focal		1 (2%)	
Interstitium, inflammation, chronic, multifocal	1 (2%)		
Interstitium, mineralization, multifocal		1 (2%)	
Nose	(49)	(50)	(50)
Foreign body	2 (4%)		
Thrombus, multifocal	2 (4%)		1 (2%)
Mucosa, erosion, multifocal			1 (2%)
Mucosa, foreign body		1 (2%)	
Mucosa, inflammation, acute			1 (2%)
Mucosa, inflammation, chronic		2 (4%)	1 (2%)
Mucosa, inflammation, chronic active	10 (20%)	7 (14%)	20 (40%)
Mucosa, inflammation, suppurative, chronic active	3 (6%)		10 (20%)
Mucosa, ulcer			1 (2%)
Mucosa, ulcer, multifocal			1 (2%)
Nasolacrimal duct, inflammation, chronic	9 (18%)	3 (6%)	1 (2%)
Nasolacrimal duct, inflammation, chronic active	3 (6%)	3 (6%)	2 (4%)
Nasolacrimal duct, inflammation, suppurative		1 (2%)	
Nasolacrimal duct, inflammation, suppurative, chronic active		2 (4%)	1 (2%)
Olfactory epithelium, atrophy			1 (2%)
Olfactory epithelium, metaplasia, squamous	1 (2%)		
Respiratory epithelium, hyperplasia	5 (10%)	2 (4%)	21 (42%)
Respiratory epithelium, hyperplasia, papillary		1 (2%)	1 (2%)
Respiratory epithelium, metaplasia, squamous			1 (2%)
Trachea	(49)	(50)	(50)
Inflammation, acute		1 (2%)	
Inflammation, chronic	3 (6%)		2 (4%)
Inflammation, chronic active		2 (4%)	2 (4%)
Inflammation, necrotizing, subacute			1 (2%)
SPECIAL SENSES SYSTEM			
Eye	(4)	(3)	
Cataract	2 (50%)	1 (33%)	
Anterior, synechia		1 (33%)	
Cornea, neovascularization, multifocal		1 (33%)	
Posterior chamber, synechia	1 (25%)		
Retina, degeneration	2 (50%)	1 (33%)	
Retina, dysplasia, focal	1 (25%)		
Lacrimal gland	(2)		
Ectopic tissue	1 (50%)		
Inflammation, chronic	1 (50%)		
Pigmentation	1 (50%)		

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	2 ppm	5 ppm
SPECIAL SENSES SYSTEM (Continued)			
Zymbal gland		(1)	
Ectasia		1 (100%)	
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Infarct			2 (4%)
Nephropathy, chronic	43 (86%)	47 (94%)	44 (88%)
Pigmentation, diffuse	9 (18%)	2 (4%)	
Pigmentation, multifocal			1 (2%)
Artery, mineralization, multifocal		1 (2%)	
Bilateral, hydronephrosis			1 (2%)
Capsule, inflammation, chronic		1 (2%)	
Medulla, mineralization, multifocal	1 (2%)		
Pelvis, epithelium, hyperplasia	1 (2%)		
Pelvis, epithelium, mineralization	1 (2%)	3 (6%)	3 (6%)
Proximal convoluted renal tubule, hyperplasia, atypical, focal		1 (2%)	
Proximal convoluted renal tubule, necrosis, acute	2 (4%)	2 (4%)	
Urinary bladder	(49)	(50)	(48)
Inflammation, acute, diffuse	1 (2%)		
Serosa, inflammation, chronic active	2 (4%)		
Transitional epithelium, hyperplasia, focal	1 (2%)		

APPENDIX C

SUMMARY OF LESIONS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	0.5 ppm	2 ppm
DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Early deaths			
Moribund sacrifice	9	19	15
Natural death	4	3	19
Accidentally killed		2	1
Survivors			
Terminal sacrifice	37	26	15
Animals examined microscopically	50	50	50
ALIMENTARY SYSTEM			
Intestine small, duodenum	(49)	(48)	(44)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Intestine small, ileum	(45)	(47)	(44)
Intestine small, jejunum	(46)	(46)	(42)
Adenocarcinoma	1 (2%)		
Liver	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	1 (2%)
Hemangioma	1 (2%)		
Hemangiosarcoma	3 (6%)	3 (6%)	1 (2%)
Hemangiosarcoma, multiple		1 (2%)	
Hepatocellular carcinoma	10 (20%)	8 (16%)	11 (22%)
Hepatocellular carcinoma, multiple	3 (6%)	3 (6%)	
Hepatocellular adenoma	7 (14%)	12 (24%)	2 (4%)
Hepatocellular adenoma, multiple	3 (6%)	3 (6%)	
Histiocytic sarcoma	1 (2%)	1 (2%)	
Mesentery		(3)	(1)
Pancreas	(50)	(49)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	2 (4%)
Duct, adenocarcinoma	1 (2%)		
Salivary glands	(50)	(48)	(50)
CARDIOVASCULAR SYSTEM			
Heart	(49)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	13 (26%)
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	3 (6%)
Subcapsular, adenoma	1 (2%)	2 (4%)	
Adrenal gland, cortex	(49)	(47)	(50)
Adenoma		1 (2%)	
Adrenal gland, medulla	(47)	(43)	(47)
Pheochromocytoma malignant	1 (2%)		
Pituitary gland	(48)	(48)	(48)
Pars distalis, adenoma			1 (2%)
Thyroid gland	(50)	(48)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Follicular cell, adenoma	2 (4%)		1 (2%)

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
GENERAL BODY SYSTEM			
Tissue, NOS		(1)	(7)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (100%)	7 (100%)
GENITAL SYSTEM			
Epididymis	(50)	(50)	(50)
Leiomyoma		1 (2%)	
Prostate	(48)	(48)	(46)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Seminal vesicle	(50)	(50)	(49)
Testes	(50)	(50)	(50)
Interstitial cell, adenoma	1 (2%)		
Tunic, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
HEMATOPOIETIC SYSTEM			
Lymph node	(50)	(50)	(50)
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Lumbar, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung			4 (8%)
Pancreatic, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Lymph node, mandibular	(37)	(39)	(43)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung			2 (5%)
Lymph node, mesenteric	(46)	(48)	(39)
Histiocytic sarcoma	2 (4%)		
Pancreatic, alveolar/bronchiolar carcinoma, metastatic, lung			1 (3%)
Spleen	(50)	(49)	(50)
Hemangiosarcoma	2 (4%)	3 (6%)	1 (2%)
Thymus	(45)	(38)	(32)
Alveolar/bronchiolar carcinoma, metastatic, lung			3 (9%)
INTEGUMENTARY SYSTEM			
Skin	(48)	(49)	(49)
Prepuce, subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Subcutaneous tissue, fibrosarcoma		1 (2%)	
Subcutaneous tissue, hemangiosarcoma		1 (2%)	
Subcutaneous tissue, lipoma		1 (2%)	
MUSCULOSKELETAL SYSTEM			
Bone	(49)	(50)	(50)
Rib, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
MUSCULOSKELETAL SYSTEM (Continued)			
Skeletal muscle			(6)
Alveolar/bronchiolar carcinoma, metastatic, lung			5 (83%)
Diaphragm, hemangiosarcoma			1 (17%)
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
RESPIRATORY SYSTEM			
Larynx	(50)	(46)	(47)
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	7 (14%)	16 (32%)	13 (26%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)	21 (42%)
Alveolar/bronchiolar carcinoma	6 (12%)	9 (18%)	6 (12%)
Alveolar/bronchiolar carcinoma, multiple		7 (14%)	40 (80%)
Hepatocellular carcinoma, metastatic, liver	5 (10%)	3 (6%)	3 (6%)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung			7 (14%)
Mediastinum, hepatocellular carcinoma, metastatic, liver	1 (2%)		
SPECIAL SENSES SYSTEM			
Harderian gland	(2)	(1)	(1)
Adenoma	2 (100%)	1 (100%)	1 (100%)
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	8 (16%)
Urinary bladder	(48)	(47)	(47)
SYSTEMIC LESIONS			
Multiple organs	*(50)	*(50)	*(50)
Histiocytic sarcoma	2 (4%)	1 (2%)	
Lymphoma malignant histiocytic		1 (2%)	
Lymphoma malignant lymphocytic		2 (4%)	1 (2%)
Lymphoma malignant mixed	1 (2%)		1 (2%)
Lymphoma malignant undifferentiated cell	1 (2%)	1 (2%)	
TUMOR SUMMARY			
Total animals with primary neoplasms**	39	40	48
Total primary neoplasms	55	79	101
Total animals with benign neoplasms	20	26	37
Total benign neoplasms	24	38	39
Total animals with malignant neoplasms	25	31	46
Total malignant neoplasms	31	41	62
Total animals with secondary neoplasms***	6	4	18
Total secondary neoplasms	6	13	65

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: CHAMBER CONTROL

DAYS ON STUDY	504	629	639	646	654	654	662	662	674	677	690	691	698	729	729	729	729	729	729	729	729	729	730	730	730
CARCASS ID	85 1	91 1	67 1	78 1	88 1	00 1	55 1	86 1	59 1	52 1	97 1	69 1	99 1	51 1	53 1	54 1	72 1	76 1	77 1	84 1	96 1	98 1	56 1	57 1	58 1
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	+	M	+	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	M	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	M	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma														X											
Hemangiosarcoma	X												X												
Hepatocellular carcinoma				X	X		X												X					X	
Hepatocellular carcinoma, multiple			X																						
Hepatocellular adenoma									X		X														
Hepatocellular adenoma, multiple																	X								X
Histiocytic sarcoma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Duct, adenocarcinoma																	X								
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																	+			+					
CARDIOVASCULAR SYSTEM																									
Blood vessel					+																				
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcapsular, adenoma																									
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	M	M	M	M	+	+	M	+	M	M	+	+	+	+	M	M	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma				X																					X
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland					+													M							
Prostate	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma							X																		

+: Tissue examined microscopically
.: Not examined
−: Present but not examined microscopically
I: Insufficient tissue

M: Missing
A: Autolysis precludes examination
X: Incidence of listed morphology

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: CHAMBER CONTROL (Continued)

DAYS ON STUDY	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
CARCASS ID	601	611	621	631	641	651	661	681	701	711	731	741	751	791	801	811	821	831	871	891	901	921	931	941	951	TOTAL: TISSUES TUMORS
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	M	M	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	M	M	+	+	40
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	46
Adenocarcinoma									X																	1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Hemangiosarcoma																				X						3
Hepatocellular carcinoma						X				X			X	X							X					10
Hepatocellular carcinoma, multiple									X														X			3
Hepatocellular adenoma		X						X								X		X	X							7
Hepatocellular adenoma, multiple																			X							3
Histiocytic sarcoma											X															1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Duct, adenocarcinoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+		+	+	+		+	+	46
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+		+	+	47
Tooth																+										3
CARDIOVASCULAR SYSTEM																										
Blood vessel																										1
Heart	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcapsular, adenoma												X														1
Adrenal gland, cortex	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, medulla	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pheochromocytoma malignant									X																	1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	M	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	M	+	M	+	M	+	33
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										2
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland		+			+																					3
Prostate	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																										1

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: CHAMBER CONTROL (Continued)

DAYS ON STUDY	504	629	639	646	654	654	662	662	674	677	690	691	698	729	729	729	729	729	729	729	729	729	730	730	730
CARCASS ID	85 1	91 1	67 1	78 1	88 1	00 1	55 1	86 1	59 1	52 1	97 1	69 1	99 1	51 1	53 1	54 1	72 1	76 1	77 1	84 1	96 1	98 1	56 1	57 1	58 1
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	M	+	+	+	M	M	+	+	M	+	+	+	M	+	+	+
Lymph node, mesenteric	M	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma		X											X												
Thymus	+	+	M	+	+	+	+	+	M	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+
INTEGUMENTARY SYSTEM																									
Mammary gland	M	M	M	M	M	+	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma							X			X							X				X	X		
Alveolar/bronchiolar carcinoma												X				X		X	X					
Hepatocellular carcinoma, metastatic, liver							X																		
Mediastinum, hepatocellular carcinoma, metastatic, liver			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Harderian gland													+										+		
Adenoma													X										X		
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymphoma malignant mixed																									
Lymphoma malignant undifferentiated cell type														X											

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: CHAMBER CONTROL (Continued)

DAYS ON STUDY	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	TOTAL:
CARCASS ID	601	611	621	631	641	651	661	681	701	711	731	741	751	791	801	811	821	831	871	891	901	921	931	941	951	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	M	M	M	+	+	M	+	M	+	M	+	+	37
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Histiocytic sarcoma							X				X															2
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										2
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	45
INTEGUMENTARY SYSTEM																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+		M	M	M	M	M	M	4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	48
MUSCULOSKELETAL SYSTEM																										
Bone	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
RESPIRATORY SYSTEM																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																			X			X				7
Alveolar/bronchiolar carcinoma									X													X				6
Hepatocellular carcinoma, metastatic, liver						X			X					X							X					5
Mediastinum, hepatocellular carcinoma, metastatic, liver																										1
Nose	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Harderian gland																										2
Adenoma																										2
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	48
SYSTEMIC LESIONS																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma							X				X															2
Lymphoma malignant mixed																						X				1
Lymphoma malignant undifferentiated cell type																										1

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: 0.5 ppm

DAYS ON STUDY	0 3 0	0 4 0	2 6 4	4 2 6	5 3 4	5 6 6	5 8 0	5 8 7	5 8 8	5 9 2	6 2 1	6 2 2	6 4 3	6 4 3	6 5 7	6 5 7	6 5 7	6 7 8	6 7 8	6 7 8	6 8 4	6 8 8	6 9 2	7 0 6	7 3 0
CARCASS ID	2 7 3 1	2 6 7 1	2 8 2 1	2 9 5 1	2 7 4 1	2 9 0 1	2 6 1 1	2 9 1 1	2 7 0 1	2 7 9 1	2 9 8 1	2 8 8 1	2 7 1 1	2 9 6 1	2 5 5 1	2 5 7 1	2 7 2 1	2 5 2 1	2 7 7 1	2 9 3 1	2 8 1 1	2 8 9 1	2 9 2 1	2 7 8 1	2 5 3 1
ALIMENTARY SYSTEM																									
Esophagus	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	M	+	+	+	+	+	+	+	+	A			+	+	+	+	M	+	A	A	+	+	M
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+
Intestine large, colon	A	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	M	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	-	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	A	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	M
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Hemangiosarcoma												X					X								
Hemangiosarcoma, multiple							X																		
Hepatocellular carcinoma			X					X		X				X		X							X		
Hepatocellular carcinoma, multiple					X																				
Hepatocellular adenoma						X	X			X							X	X	X						
Hepatocellular adenoma, multiple																									
Histiocytic sarcoma				X																					
Mesentery																				+		+			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Salivary glands	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth															+										
CARDIOVASCULAR SYSTEM																									
Blood vessel																							+		
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung						X																			
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Subcapsular, adenoma																			X						
Adrenal gland, cortex	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal gland, medulla	M	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	M	+	+	M	+	+	+	+	+	+	+	+	M	M	+	M	M	+	+	M	M	+	M	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GENERAL BODY SYSTEM																									
Tissue, NOS						+																			
Alveolar/bronchiolar carcinoma, metastatic, lung						X																			
GENITAL SYSTEM																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma			X																						
Penis								+																	
Preputial gland			+			+																	+		
Prostate	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tunic, alveolar/bronchiolar carcinoma, metastatic, lung						X																			

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: 0.5 ppm (Continued)

DAYS ON STUDY	730	730	730	730	730	730	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	TOTAL:
CARCASS ID	25401	25601	25801	25901	26001	26201	26301	26401	26501	26601	25111	26801	26901	27551	27601	28001	28301	28401	28551	28601	28711	29401	29701	29901	30001	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Gallbladder	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	39
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	46
Intestine large, rectum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Hemangiosarcoma																							X			3
Hemangiosarcoma, multiple																										1
Hepatocellular carcinoma													X										X			8
Hepatocellular carcinoma, multiple																			X	X						3
Hepatocellular adenoma				X	X						X	X	X												X	12
Hepatocellular adenoma, multiple			X					X								X										3
Histiocytic sarcoma																										1
Mesentery																				+						3
Pancreas	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth		+																								2
CARDIOVASCULAR SYSTEM																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Subcapsular, adenoma																					X					2
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma									X																	1
Adrenal gland, medulla	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	43
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	M	M	+	M	+	+	+	M	+	+	M	+	M	+	+	+	+	+	M	+	+	M	+	M	30
Pituitary gland	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
GENERAL BODY SYSTEM																										
Tissue, NOS																										1
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma																										1
Penis																										1
Preputial gland														+												4
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tunic, alveolar/bronchiolar carcinoma, metastatic, lung																										1

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: 0.5 ppm (Continued)

DAYS ON STUDY	030	048	264	426	534	566	580	587	588	592	621	622	643	643	657	657	657	678	678	678	684	688	692	706	730
CARCASS ID	27311	26711	28211	29511	27411	29011	26111	29111	27011	27911	29811	28811	27111	29611	25511	25711	27211	25211	27711	29311	28111	28911	29211	27811	25311
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreatic, alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Lymph node, mandibular	M	M	M	+	+		+	+	+	+	+	+	M	+	+	M	+	+	M	M	+	+	+	+	+
Lymph node, mesenteric	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma												X													
Thymus	M	+	M	M	+	+	+	M	M	+	+	M	+	+	+	M	M	+	+	+	M	+	M	+	+
INTEGUMENTARY SYSTEM																									
Mammary gland	M	M	+	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	+	M	M
Skin	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prepuce, subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Subcutaneous tissue, fibrosarcoma																									
Subcutaneous tissue, *hemangiosarcoma*																									
Subcutaneous tissue, lipoma																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Larynx	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X												X		X	X				X	
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma											X				X									X	
Alveolar/bronchiolar carcinoma, multiple						X							X					X			X		X		
Hepatocellular carcinoma, metastatic, liver					X																		X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	M	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Eye																									
Harderian gland																									
Adenoma																									
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Urinary bladder	M	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	A	+	+	+	+
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X																					
Lymphoma malignant histiocytic														X											
Lymphoma malignant lymphocytic																				X		X			
Lymphoma malignant undifferentiated cell type																X									

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: 0.5 ppm (Continued)

DAYS ON STUDY	730	730	730	730	730	730	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	TOTAL:
CARCASS ID	25401	25611	25811	25911	26001	26021	26031	26401	26501	26611	25101	26801	26901	27511	27611	28001	28311	28411	28511	28611	28711	29411	29711	29911	30011	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreatic, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Lymph node, mandibular	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	39
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma		X											X													3
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	38
INTEGUMENTARY SYSTEM																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Prepuce, subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Subcutaneous tissue, fibrosarcoma																							X			1
Subcutaneous tissue, hemangiosarcoma																						X				1
Subcutaneous tissue, lipoma																					X					1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
RESPIRATORY SYSTEM																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X	X							X	X	X		X		X		X		X			X	X	16
Alveolar/bronchiolar adenoma, multiple																				X						1
Alveolar/bronchiolar carcinoma				X				X				X	X			X								X		9
Alveolar/bronchiolar carcinoma, multiple																				X		X				7
Hepatocellular carcinoma, metastatic, liver																				X						3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
SPECIAL SENSES SYSTEM																										
Eye													+													1
Harderian gland													+													1
Adenoma													X													1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
SYSTEMIC LESIONS																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic																										2
Lymphoma malignant undifferentiated cell type																										1

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: 2 ppm

DAYS ON STUDY	1 4 5	2 9 9	3 7 6	4 8 5	5 1 3	5 1 4	5 3 7	5 4 6	5 5 3	5 5 5	5 6 0	5 6 5	5 7 1	5 8 8	5 9 1	6 0 4	6 1 4	6 1 4	6 2 5	6 2 7	6 4 2	6 4 2	6 4 9	6 5 2	6 5 4
CARCASS ID	1 8 4 1	1 6 4 1	1 7 7 1	1 6 3 1	2 0 0 1	1 9 5 1	1 9 4 1	1 8 1 1	1 7 5 1	1 7 4 1	1 5 1 1	1 9 9 1	1 7 9 1	1 6 8 1	1 7 0 1	1 8 5 1	1 9 0 1	1 9 7 1	1 7 1 1	1 9 3 1	1 6 2 1	1 8 3 1	1 5 7 1	1 7 8 1	1 9 8 1
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	M	M	+	A	A	+	A	A	+	M	+	A	M	+	A	+	+	+	+	+	+	+		A
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	M	+	+	+	+	A	A	+	+	+	M	A	+	+	+	+	+	+	+	+	+	A	+
Intestine large, colon	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	M	M	+	M	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A
Alveolar/bronchiolar carcinoma, metastatic, lung													X												
Intestine small, ileum	M	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	M	+	+	+	+	+	+	+	+	+	A	+	A	+	+	+	+	+	+		+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Hemangiosarcoma																									
Hepatocellular carcinoma								X																	
Hepatocellular adenoma																									
Mesentery																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																									
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X		X			X	X			X		X	X			X				X	X	X
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X					X															X	
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M		+
Islets, pancreatic	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Parathyroid gland	M	+	M	+	+	+	+	M	+	+	M	+	+	+	+	M	+	+	+	M	M	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X																								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Follicular cell, adenoma																									
GENERAL BODY SYSTEM																									
Tissue, NOS						+			+				+			+								+	
Alveolar/bronchiolar carcinoma, metastatic, lung						X			X				X			X								X	
GENITAL SYSTEM																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis								+																	
Preputial gland			+				+																		
Prostate	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: 2 ppm
(Continued)

DAYS ON STUDY	659	671	684	691	710	714	717	717	718	722	729	729	729	729	729	729	729	729	729	730	730	730	730	730	
CARCASS ID	18881	15611	16511	18891	19211	16011	17611	18611	16711	19111	15211	15311	15411	15911	16111	16611	16911	17211	17311	18011	15511	15811	18211	18711	19061	TOTAL: TISSUES TUMORS
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	M	M	A	+	+	M	+		M	+	+	+	+	+	+	+	+	M	+	+	+	+	31
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Intestine large, colon	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Intestine small, ileum	+	M	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	44
Intestine small, jejunum	+	A	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	42
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Hemangiosarcoma																										1
Hepatocellular carcinoma				X			X		X			X						X				X				11
Hepatocellular adenoma		X		X																						2
Mesentery								+																		1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung			X						X																	2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth								+					+	+	+	+	+	+					+			8
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung			X						X																	13
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										3
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	M	+	M	M	+	+	+	+	+	+	M	M	M	+	+	M	M	+	+	M	34
Pituitary gland	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Follicular cell, adenoma								X																		1
GENERAL BODY SYSTEM																										
Tissue, NOS			+						+																	7
Alveolar/bronchiolar carcinoma, metastatic, lung			X						X																	7
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																					+					2
Preputial gland																					+				+	4
Prostate	+	+	+	+	−	+	+		M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	46
Seminal vesicle	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: 2 ppm (Continued)

DAYS ON STUDY	145	299	376	485	513	514	537	548	553	555	560	565	571	588	591	604	614	614	625	627	642	642	649	652	654
CARCASS ID	18411	16411	17711	16311	20011	19511	19411	18111	17511	17411	15111	19911	17911	16811	17011	18511	19011	19711	17111	19311	16211	18311	15711	17811	19811
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung																							X		
Lumbar, alveolar/bronchiolar carcinoma, metastatic, lung																									
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung									X	X						X									X
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung															X										
Lymph node, mesenteric	M	M	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+
Pancreatic, alveolar/bronchiolar carcinoma, metastatic, lung													X												
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	M	+	M	+	+	M	+	+	+	+	+	M	M	M	M	+	+	+	+	+		+	M
Alveolar/bronchiolar carcinoma, metastatic, lung									X				X												
INTEGUMENTARY SYSTEM																									
Mammary gland	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, *alveolar/bronchiolar carcinoma*, metastatic, lung															X										
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rib, alveolar/bronchiolar carcinoma, metastatic, lung																			X						
Skeletal muscle									+					+	+										+
Alveolar/bronchiolar carcinoma, metastatic, lung									X						X										X
Diaphragm, hemangiosarcoma														X											
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord												+													
RESPIRATORY SYSTEM																									
Larynx	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X					X				X				X			X			X
Alveolar/bronchiolar adenoma, multiple			X				X	X				X	X	X		X	X	X			X			X	
Alveolar/bronchiolar carcinoma				X			X	X			X											X			
Alveolar/bronchiolar carcinoma, multiple						X			X	X		X	X	X	X	X	X	X	X	X	X		X	X	X
Hepatocellular carcinoma, metastatic, liver								X											X						
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung				X					X	X													X		X
Nose	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Harderian gland																									
Adenoma																									
Lacrimal gland																									
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X									X										X	X	X
Urinary bladder	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																									
Lymphoma malignant mixed																									

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: 2 ppm (Continued)

DAYS ON STUDY	659	671	684	691	710	714	717	717	718	722	729	729	729	729	729	729	729	729	729	730	730	730	730	730	TOTAL: TISSUES TUMORS
CARCASS ID	18881	15661	16651	18991	19211	16011	17661	18611	16711	19111	15211	15311	15411	15911	16111	16661	16991	17211	17311	18011	15551	15811	18211	18711	19061	
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Lumbar, alveolar/bronchiolar carcinoma, metastatic, lung			X																							1
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										4
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	43
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung									X																	2
Lymph node, mesenteric	+	M	+	+	+		M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+		+	+	39
Pancreatic, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																		X								1
Thymus	+	M	+	M	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	M	M	M	+	M	32
Alveolar/bronchiolar carcinoma, metastatic, lung			X																							3
INTEGUMENTARY SYSTEM																									
Mammary gland	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	49
Subcutaneous tissue, alveolar/bronchiolar carcinoma, metastatic, lung																										1
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Rib, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Skeletal muscle			+						+																	6
Alveolar/bronchiolar carcinoma, metastatic, lung			X						X																	5
Diaphragm, hemangiosarcoma																										1
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										1
RESPIRATORY SYSTEM																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	47
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X					X			X							X			X			X		X		13
Alveolar/bronchiolar adenoma, multiple				X			X					X	X		X		X	X		X	X		X			21
Alveolar/bronchiolar carcinoma																X										6
Alveolar/bronchiolar carcinoma, multiple	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	40
Hepatocellular carcinoma, metastatic, liver												X														3
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung															X							X				7
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	49
SPECIAL SENSES SYSTEM																									
Harderian gland																		+								1
Adenoma																		X								1
Lacrimal gland																	•									1
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung			X						X																X	8
Urinary bladder	+	+	+	+	+	A	+	+	+	+		+	+	+	+	+	+	+	+	•	+	•	+	+	+	47
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic								X																		1
Lymphoma malignant mixed																			X							1

TABLE C3. ANALYSIS OF PRIMARY NEOPLASMS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	0.5 ppm	2 ppm
Liver: Hepatocellular Adenoma			
Overall Rates (a)	10/50 (20%)	15/50 (30%)	2/50 (4%)
Adjusted Rates (b)	25.4%	44.3%	8.5%
Terminal Rates (c)	8/37 (22%)	9/26 (35%)	0/15 (0%)
Day of First Observation	674	566	671
Life Table Tests (d)	P=0.149N	P=0.042	P=0.208N
Logistic Regression Tests (d)	P=0.017N	P=0.103	P=0.076N
Cochran-Armitage Trend Test (d)	P=0.005N		
Fisher Exact Test (d)		P=0.178	P=0.014N
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	13/50 (26%)	11/50 (22%)	11/50 (22%)
Adjusted Rates (b)	30.7%	29.5%	41.0%
Terminal Rates (c)	9/37 (24%)	4/26 (15%)	3/15 (20%)
Day of First Observation	639	264	546
Life Table Tests (d)	P=0.143	P=0.470	P=0.132
Logistic Regression Tests (d)	P=0.429N	P=0.352N	P=0.547N
Cochran-Armitage Trend Test (d)	P=0.418N		
Fisher Exact Test (d)		P=0.408N	P=0.408N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	23/50 (46%)	24/50 (48%)	12/50 (24%)
Adjusted Rates (b)	52.9%	60.9%	43.5%
Terminal Rates (c)	17/37 (46%)	12/26 (46%)	3/15 (20%)
Day of First Observation	639	264	546
Life Table Tests (d)	P=0.540N	P=0.118	P=0.491
Logistic Regression Tests (d)	P=0.015N	P=0.443	P=0.076N
Cochran-Armitage Trend Test (d)	P=0.007N		
Fisher Exact Test (d)		P=0.500	P=0.018N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	7/50 (14%)	17/50 (34%)	34/50 (68%)
Adjusted Rates (b)	17.5%	53.7%	90.5%
Terminal Rates (c)	5/37 (14%)	12/26 (46%)	12/15 (80%)
Day of First Observation	662	534	376
Life Table Tests (d)	P<0.001	P=0.002	P<0.001
Logistic Regression Tests (d)	P<0.001	P=0.004	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.017	P<0.001
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	6/50 (12%)	16/50 (32%)	46/50 (92%)
Adjusted Rates (b)	15.7%	45.6%	100.0%
Terminal Rates (c)	5/37 (14%)	8/26 (31%)	15/15 (100%)
Day of First Observation	691	566	485
Life Table Tests (d)	P<0.001	P=0.002	P<0.001
Logistic Regression Tests (d)	P<0.001	P=0.006	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.014	P<0.001
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	12/50 (24%)	27/50 (54%)	47/50 (94%)
Adjusted Rates (b)	29.7%	70.4%	100.0%
Terminal Rates (c)	9/37 (24%)	15/26 (58%)	15/15 (100%)
Day of First Observation	662	534	376
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Logistic Regression Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.002	P<0.001

TABLE C3. ANALYSIS OF PRIMARY NEOPLASMS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
Circulatory System: Hemangiosarcoma			
Overall Rates (e)	4/50 (8%)	7/50 (14%)	2/50 (4%)
Adjusted Rates (b)	9.1%	21.7%	9.2%
Terminal Rates (c)	1/37 (3%)	4/26 (15%)	1/15 (7%)
Day of First Observation	504	580	588
Life Table Tests (d)	P=0.498N	P=0.141	P=0.594N
Logistic Regression Tests (d)	P=0.186N	P=0.285	P=0.235N
Cochran-Armitage Trend Test (d)	P=0.189N		
Fisher Exact Test (d)		P=0.262	P=0.339N
Circulatory System: Hemangioma or Hemangiosarcoma			
Overall Rates (e)	5/50 (10%)	7/50 (14%)	2/50 (4%)
Adjusted Rates (b)	11.6%	21.7%	9.2%
Terminal Rates (c)	2/37 (5%)	4/26 (15%)	1/15 (7%)
Day of First Observation	504	580	588
Life Table Tests (d)	P=0.422N	P=0.215	P=0.497N
Logistic Regression Tests (d)	P=0.133N	P=0.398	P=0.156N
Cochran-Armitage Trend Test (d)	P=0.130N		
Fisher Exact Test (d)		P=0.380	P=0.218N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (e)	2/50 (4%)	4/50 (8%)	2/50 (4%)
Adjusted Rates (b)	5.4%	11.4%	11.6%
Terminal Rates (c)	2/37 (5%)	0/26 (0%)	1/15 (7%)
Day of First Observation	729	643	717
Life Table Tests (d)	P=0.433	P=0.227	P=0.372
Logistic Regression Tests (d)	P=0.626N	P=0.315	P=0.437
Cochran-Armitage Trend Test (d)	P=0.514N		
Fisher Exact Test (d)		P=0.339	P=0.691N
All Sites: Benign Tumors			
Overall Rates (e)	20/50 (40%)	26/50 (52%)	37/50 (74%)
Adjusted Rates (b)	46.2%	70.8%	91.6%
Terminal Rates (c)	14/37 (38%)	16/26 (62%)	12/15 (80%)
Day of First Observation	646	264	145
Life Table Tests (d)	P<0.001	P=0.017	P<0.001
Logistic Regression Tests (d)	P<0.001	P=0.092	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.158	P<0.001
All Sites: Malignant Tumors			
Overall Rates (e)	25/50 (50%)	31/50 (62%)	46/50 (92%)
Adjusted Rates (b)	54.9%	66.9%	100.0%
Terminal Rates (c)	17/37 (46%)	11/26 (42%)	15/15 (100%)
Day of First Observation	504	264	485
Life Table Tests (d)	P<0.001	P=0.026	P<0.001
Logistic Regression Tests (d)	P<0.001	P=0.188	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.157	P<0.001
All Sites: All Tumors			
Overall Rates (e)	39/50 (78%)	40/50 (80%)	48/50 (96%)
Adjusted Rates (b)	81.2%	85.1%	100.0%
Terminal Rates (c)	28/37 (76%)	19/26 (73%)	15/15 (100%)
Day of First Observation	504	264	145
Life Table Tests (d)	P<0.001	P=0.039	P<0.001
Logistic Regression Tests (d)	P=0.002	P=0.366	P=0.004
Cochran-Armitage Trend Test (d)	P=0.006		
Fisher Exact Test (d)		P=0.500	P=0.007

TABLE C3. ANALYSIS OF PRIMARY NEOPLASMS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

(a) Number of tumor-bearing animals/number of animals examined microscopically at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors 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. A negative trend or lower incidence in a dosed group than in controls is indicated by (N).
(e) Number of tumor-bearing animals/number of animals examined grossly at the site

TABLE C4. HISTORICAL INCIDENCE OF ALVEOLAR/BRONCHIOLAR NEOPLASMS IN MALE B6C3F$_1$ MICE (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence for Chamber Controls in NTP Studies (b)			
Propylene oxide	14/50	2/50	15/50
Methyl methacrylate	10/50	3/50	11/50
Propylene	7/50	9/50	16/50
1,2-Epoxybutane	7/49	5/49	11/49
Dichloromethane	3/50	2/50	5/50
Ethylene oxide	5/50	6/50	11/50
Bromoethane	5/50	2/50	7/50
Tetrachloroethylene	3/49	4/49	6/49
TOTAL	54/398 (13.6%)	33/398 (8.3%)	82/398 (20.6%)
SD (c)	7.45%	4.96%	8.03%
Range (d)			
High	14/50	9/50	16/50
Low	3/50	2/50	5/50
Overall Historical Incidence for Untreated Controls in NTP Studies			
TOTAL	204/1,684 (12.1%)	80/1,684 (4.8%)	277/1,684 (16.4%)
SD (c)	6.18%	2.70%	6.91%
Range (d)			
High	14/50	5/49	17/50
Low	1/50	0/49	4/50

(a) Data as of March 1, 1989, for studies of at least 104 weeks
(b) All inhalation studies included in the NTP historical data base were conducted at Battelle Pacific Northwest Laboratories.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	0.5 ppm	2 ppm
DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Early deaths			
Moribund sacrifice	9	19	15
Natural death	4	3	19
Accidentally killed		2	1
Survivors			
Terminal sacrifice	37	26	15
Animals examined microscopically	50	50	50
ALIMENTARY SYSTEM			
Gallbladder	(40)	(39)	(31)
Ectasia		1 (3%)	
Infiltration cellular, lymphocytic, focal			2 (6%)
Infiltration cellular, lymphocytic, multifocal	2 (5%)	3 (8%)	
Intestine large, cecum	(48)	(47)	(40)
Peyer's patch, hyperplasia, lymphoid	6 (13%)	3 (6%)	
Intestine large, colon	(49)	(46)	(48)
Infiltration cellular, lymphocytic, multifocal			1 (2%)
Intestine small, duodenum	(49)	(48)	(44)
Ectopic tissue		1 (2%)	
Lumen, hemorrhage, acute	1 (2%)		
Intestine small, ileum	(45)	(47)	(44)
Peyer's patch, hyperplasia, lymphoid	1 (2%)		
Intestine small, jejunum	(46)	(46)	(42)
Hyperplasia			1 (2%)
Lumen, hemorrhage, acute	1 (2%)		
Liver	(50)	(50)	(50)
Angiectasis, multifocal		1 (2%)	
Basophilic focus	2 (4%)	2 (4%)	2 (4%)
Basophilic focus, multiple	1 (2%)		
Cytomegaly, multifocal	1 (2%)		
Cytoplasmic alteration, multifocal			1 (2%)
Eosinophilic focus	1 (2%)		1 (2%)
Fatty change, focal	1 (2%)		
Fatty change, multifocal		1 (2%)	
Hematopoietic cell proliferation, multifocal	1 (2%)	2 (4%)	2 (4%)
Hepatodiaphragmatic nodule	1 (2%)		1 (2%)
Hyperplasia, focal		1 (2%)	
Hyperplasia, nodular, multifocal	1 (2%)		
Infarct	2 (4%)	5 (10%)	2 (4%)
Infiltration cellular, lymphocytic, focal		1 (2%)	1 (2%)
Infiltration cellular, lymphocytic, multifocal	4 (8%)	1 (2%)	3 (6%)
Inflammation, chronic, multifocal	1 (2%)	3 (6%)	2 (4%)
Inflammation, granulomatous, multifocal		1 (2%)	
Inflammation, subacute, multifocal	2 (4%)	4 (8%)	
Mitotic alteration		1 (2%)	
Mixed cell focus	1 (2%)		
Necrosis, acute, multifocal	3 (6%)	3 (6%)	2 (4%)
Necrosis, chronic, multifocal		1 (2%)	
Pigmentation, multifocal		1 (2%)	
Thrombus			1 (2%)
Artery, mineralization, multifocal		1 (2%)	
Bile duct, hyperplasia, multifocal	4 (8%)	3 (6%)	3 (6%)
Centrilobular, fatty change, multifocal	2 (4%)		1 (2%)
Centrilobular, necrosis, acute, multifocal			1 (2%)
Centrilobular, necrosis, diffuse	1 (2%)		1 (2%)
Hepatocyte, atrophy, multifocal		1 (2%)	
Kupffer cell, hyperplasia, multifocal			1 (2%)
Oval cell, hyperplasia	1 (2%)		

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
ALIMENTARY SYSTEM (Continued)			
Mesentery		(3)	(1)
Angiectasis, multifocal		1 (33%)	
Fat, inflammation, chronic		1 (33%)	
Pancreas	(50)	(49)	(49)
Hyperplasia, focal	1 (2%)		
Artery, inflammation, chronic active			1 (2%)
Duct, cyst	1 (2%)		
Duct, dilatation			1 (2%)
Salivary glands	(50)	(48)	(50)
Infiltration cellular, lymphocytic, multifocal	25 (50%)	21 (44%)	7 (14%)
Stomach, forestomach	(46)	(49)	(50)
Inflammation, chronic active, focal	1 (2%)		
Ulcer	1 (2%)		
Epithelium, hyperplasia, diffuse	1 (2%)		
Epithelium, hyperplasia, focal			1 (2%)
Stomach, glandular	(47)	(49)	(49)
Inflammation, acute, focal			1 (2%)
Inflammation, chronic			1 (2%)
Mucosa, mineralization	1 (2%)		1 (2%)
Tooth	(3)	(2)	(8)
Abscess		1 (50%)	
Dysplasia	2 (67%)	2 (100%)	5 (63%)
Inflammation, acute			1 (13%)
Inflammation, chronic			2 (25%)
Inflammation, chronic active	1 (33%)		3 (38%)
CARDIOVASCULAR SYSTEM			
Blood vessel	(1)	(1)	
Inflammation, chronic		1 (100%)	
Artery, inflammation, chronic, multifocal	1 (100%)		
Mesenteric artery, inflammation, chronic		1 (100%)	
Mesenteric artery, thrombus		1 (100%)	
Renal artery, inflammation, chronic, multifocal		1 (100%)	
Heart	(49)	(50)	(50)
Cardiomyopathy		2 (4%)	
Inflammation, acute, multifocal			1 (2%)
Inflammation, chronic, focal		1 (2%)	
Inflammation, chronic, multifocal			1 (2%)
Aortic valve, inflammation, chronic active, focal		1 (2%)	
Epicardium, hyperplasia, focal		1 (2%)	
Mitral valve, bacterium			1 (2%)
Mitral valve, inflammation, subacute, focal		1 (2%)	
Perivascular, granuloma		1 (2%)	
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(49)	(50)
Capsule, accessory adrenal cortical nodule			3 (6%)
Subcapsular, hyperplasia, focal	4 (8%)	4 (8%)	3 (6%)
Subcapsular, hyperplasia, multifocal	32 (64%)	27 (55%)	28 (56%)
Adrenal gland, cortex	(49)	(47)	(50)
Cyst		1 (2%)	
Hyperplasia, focal	5 (10%)	7 (15%)	4 (8%)
Hyperplasia, multifocal	1 (2%)	3 (6%)	
Hypertrophy, focal	6 (12%)	3 (6%)	7 (14%)
Hypertrophy, multifocal	6 (12%)	3 (6%)	1 (2%)
Adrenal gland, medulla	(47)	(43)	(47)
Hyperplasia, focal		1 (2%)	1 (2%)
Hyperplasia, multifocal		1 (2%)	

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
ENDOCRINE SYSTEM (Continued)			
Islets, pancreatic	(50)	(49)	(48)
Hyperplasia, focal	1 (2%)	2 (4%)	
Hyperplasia, multifocal	18 (36%)	7 (14%)	23 (48%)
Pituitary gland	(48)	(48)	(48)
Pars distalis, cyst	1 (2%)	1 (2%)	
Pars distalis, cyst, multiple		1 (2%)	
Pars distalis, hyperplasia, focal	1 (2%)		
Thyroid gland	(50)	(48)	(50)
Inflammation, chronic, focal	1 (2%)		
Inflammation, chronic, multifocal	1 (2%)		
Follicle, cyst		1 (2%)	2 (4%)
Follicle, cyst, multiple	3 (6%)		2 (4%)
Follicular cell, hyperplasia, focal	1 (2%)		2 (4%)
Follicular cell, hyperplasia, multifocal	3 (6%)		
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Epididymis	(50)	(50)	(50)
Granuloma		1 (2%)	
Granuloma sperm		1 (2%)	
Inflammation, chronic, multifocal			1 (2%)
Penis		(1)	(2)
Inflammation, chronic active			2 (100%)
Preputial gland	(3)	(4)	(4)
Abscess		2 (50%)	
Atrophy			2 (50%)
Ectasia	2 (67%)	1 (25%)	3 (75%)
Inflammation, chronic	1 (33%)	1 (25%)	2 (50%)
Prostate	(48)	(48)	(46)
Ectasia, multifocal	1 (2%)	1 (2%)	
Granuloma		1 (2%)	
Infiltration cellular, lymphocytic, multifocal		1 (2%)	
Inflammation, chronic	1 (2%)		
Inflammation, chronic active	1 (2%)	1 (2%)	2 (4%)
Inflammation, suppurative, acute			1 (2%)
Serosa, inflammation, suppurative, acute, focal		1 (2%)	
Seminal vesicle	(50)	(50)	(49)
Abscess			1 (2%)
Ectasia		3 (6%)	
Inflammation, chronic			1 (2%)
Testes	(50)	(50)	(50)
Interstitial cell, hyperplasia, diffuse		2 (4%)	
Seminiferous tubule, atrophy, multifocal		8 (16%)	
Seminiferous tubule, degeneration, multifocal		1 (2%)	
HEMATOPOIETIC SYSTEM			
Bone marrow	(48)	(50)	(49)
Hyperplasia, re cell, focal			1 (2%)
Metaplasia, osseous, focal		1 (2%)	
Myeloid cell, hyperplasia		1 (2%)	2 (4%)
Lymph node	(50)	(50)	(50)
Bronchial, hyperplasia, lymphoid			1 (2%)
Inguinal, hyperplasia		1 (2%)	
Inguinal, hyperplasia, lymphoid	3 (6%)		
Inguinal, hyperplasia, plasma cell			1 (2%)
Mediastinal, hyperplasia			3 (6%)

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
HEMATOPOIETIC SYSTEM			
Lymph node (Continued)	(50)	(50)	(50)
Mediastinal, hyperplasia, lymphoid	2 (4%)		1 (2%)
Mediastinal, hyperplasia, plasma cell			1 (2%)
Lymph node, mandibular	(37)	(39)	(43)
Hyperplasia	1 (3%)		
Hyperplasia, lymphoid	2 (5%)	1 (3%)	2 (5%)
Hyperplasia, re cell			2 (5%)
Pigmentation, hemosiderin	1 (3%)	1 (3%)	3 (7%)
Lymph node, mesenteric	(46)	(48)	(39)
Angiectasis			1 (3%)
Hematopoietic cell proliferation	13 (28%)	7 (15%)	5 (13%)
Hemorrhage, acute	27 (59%)	25 (52%)	19 (49%)
Hemorrhage, subacute	5 (11%)	3 (6%)	1 (3%)
Hyperplasia, lymphoid	3 (7%)	4 (8%)	4 (10%)
Hyperplasia, re cell			1 (3%)
Pigmentation, hemosiderin		1 (2%)	
Spleen	(50)	(49)	(50)
Angiectasis, multifocal			1 (2%)
Fibrosis, diffuse			1 (2%)
Hematopoietic cell proliferation	18 (36%)	10 (20%)	8 (16%)
Hyperplasia, lymphoid	3 (6%)	1 (2%)	2 (4%)
Hyperplasia, re cell			1 (2%)
Necrosis, subacute, focal	1 (2%)		
Thymus	(45)	(38)	(32)
Cyst	1 (2%)		
Depletion lymphoid	2 (4%)		6 (19%)
Hyperplasia, lymphoid	1 (2%)		1 (3%)
Necrosis	1 (2%)		
INTEGUMENTARY SYSTEM			
Skin	(48)	(49)	(49)
Prepuce, inflammation, chronic active	2 (4%)	1 (2%)	3 (6%)
Prepuce, ulcer	1 (2%)	1 (2%)	3 (6%)
Subcutaneous tissue, abscess, chronic			1 (2%)
Subcutaneous tissue, edema	1 (2%)	1 (2%)	
Subcutaneous tissue, inflammation, granulomatous, focal	1 (2%)		
Subcutaneous tissue, inflammation, subacute, diffuse			1 (2%)
Subcutaneous tissue, inflammation, subacute, focal		1 (2%)	
MUSCULOSKELETAL SYSTEM			
Bone	(49)	(50)	(50)
Fibrous osteodystrophy	3 (6%)		
Osteoporosis			1 (2%)
Sternum, developmental malformation		2 (4%)	
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
Compression			1 (2%)
Hemorrhage, acute, multifocal			1 (2%)
Infarct, subacute			1 (2%)
Inflammation, acute, focal			1 (2%)
Mineralization, multifocal	31 (62%)	29 (58%)	30 (60%)

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
RESPIRATORY SYSTEM			
Larynx	(50)	(46)	(47)
Inflammation, chronic	1 (2%)	1 (2%)	
Inflammation, chronic active		2 (4%)	
Lung	(50)	(50)	(50)
Hemorrhage, acute	2 (4%)	1 (2%)	4 (8%)
Hemorrhage, subacute		1 (2%)	
Infiltration cellular, lymphocytic, multifocal	4 (8%)	1 (2%)	1 (2%)
Inflammation, acute, focal			1 (2%)
Necrosis, acute, multifocal			2 (4%)
Thrombus		1 (2%)	
Alveolar epithelium, hyperplasia, focal	2 (4%)	13 (26%)	1 (2%)
Alveolar epithelium, hyperplasia, multifocal		8 (16%)	45 (90%)
Alveolus, infiltration cellular, histiocytic, diffuse		1 (2%)	2 (4%)
Alveolus, infiltration cellular, histiocytic, focal	1 (2%)		
Alveolus, infiltration cellular, histiocytic, multifocal	6 (12%)	4 (8%)	20 (40%)
Artery, thrombus, multifocal		1 (2%)	
Bronchiole, hyperplasia, focal		4 (8%)	
Bronchiole, hyperplasia, multifocal		5 (10%)	40 (80%)
Bronchiole, alveolus, inflammation, suppurative, acute, multifocal			1 (2%)
Mediastinum, infiltration cellular, lymphocytic, multifocal	1 (2%)		1 (2%)
Nose	(49)	(50)	(49)
Lumen, exudate	1 (2%)	1 (2%)	29 (59%)
Mucosa, inflammation, acute			1 (2%)
Mucosa, inflammation, chronic active	1 (2%)	2 (4%)	5 (10%)
Nasolacrimal duct, exudate			1 (2%)
Nasolacrimal duct, hyperplasia			1 (2%)
Nasolacrimal duct, inflammation, acute			1 (2%)
Nasolacrimal duct, inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)
Olfactory epithelium, atrophy			1 (2%)
Respiratory epithelium, hyperplasia	3 (6%)	6 (12%)	5 (10%)
Respiratory epithelium, ulcer, focal			1 (2%)
Trachea	(50)	(47)	(49)
Inflammation, chronic active	2 (4%)		
SPECIAL SENSES SYSTEM			
Eye		(1)	
Atrophy		1 (100%)	
Lacrimal gland			(1)
Infiltration cellular, lymphocytic, multifocal			1 (100%)
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Abscess			1 (2%)
Abscess, multiple			1 (2%)
Hydronephrosis	1 (2%)	3 (6%)	2 (4%)
Infarct		1 (2%)	3 (6%)
Infiltration cellular, plasma cell		1 (2%)	
Infiltration cellular, lymphocytic	18 (36%)	15 (30%)	7 (14%)
Mineralization, multifocal	2 (4%)		1 (2%)
Nephropathy, chronic	3 (6%)	5 (10%)	
Bilateral, hydronephrosis	2 (4%)		
Bilateral, pelvis, inflammation, acute	2 (4%)		1 (2%)
Capsule, fibrosis, focal			1 (2%)
Cortex, cyst		1 (2%)	

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
URINARY SYSTEM			
Kidney (Continued)	(50)	(50)	(50)
Medulla, necrosis, acute, focal	1 (2%)		
Pelvis, inflammation, acute		1 (2%)	
Proximal convoluted renal tubule, necrosis, acute, multifocal	1 (2%)		
Renal tubule, cytoplasmic alteration, multifocal		1 (2%)	
Renal tubule, hyperplasia, atypical, focal		2 (4%)	1 (2%)
Renal tubule, hyperplasia, focal			1 (2%)
Renal tubule, hyperplasia, multifocal	1 (2%)	2 (4%)	
Urinary bladder	(48)	(47)	(47)
Infiltration cellular, lymphocytic, multifocal		1 (2%)	1 (2%)
Inflammation, chronic, diffuse	1 (2%)		1 (2%)
Inflammation, chronic, multifocal	1 (2%)		
Inflammation, chronic active, diffuse	2 (4%)		
Ulcer, multifocal	1 (2%)		
Transitional epithelium, hyperplasia, atypical, diffuse	1 (2%)		

APPENDIX D

SUMMARY OF LESIONS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	0.5 ppm	2 ppm
DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Early deaths			
Natural death	5	4	10
Accidentally killed	2	1	
Moribund sacrifice	12	17	16
Survivors			
Terminal sacrifice	30	27	23
Natural death	1		
Moribund sacrifice		1	1
Animals examined microscopically	50	50	50
ALIMENTARY SYSTEM			
Esophagus	(49)	(49)	(49)
Gallbladder	(42)	(40)	(36)
Intestine large, cecum	(47)	(44)	(45)
Intestine large, colon	(49)	(47)	(46)
Intestine large, rectum	(46)	(48)	(48)
Liver	(49)	(50)	(50)
Hemangiosarcoma			1 (2%)
Hemangiosarcoma, metastatic, spleen			2 (4%)
Hepatocellular carcinoma	4 (8%)	2 (4%)	3 (6%)
Hepatocellular adenoma	8 (16%)	1 (2%)	4 (8%)
Hepatocellular adenoma, multiple	1 (2%)		
Mesentery	(3)	(1)	(5)
Pancreas	(49)	(50)	(50)
Salivary glands	(48)	(50)	(50)
Stomach, forestomach	(48)	(50)	(49)
Papilloma squamous		1 (2%)	
Stomach, glandular	(47)	(49)	(49)
Tooth	(2)		(2)
Peridontal tissue, alveolar/bronchiolar carcinoma, metastatic, lung			1 (50%)
CARDIOVASCULAR SYSTEM			
Heart	(47)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	5 (10%)
ENDOCRINE SYSTEM			
Adrenal gland	(49)	(49)	(50)
Extra adrenal tissue, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Subcapsular, adenoma			1 (2%)
Adrenal gland, cortex	(49)	(48)	(50)
Adenoma	1 (2%)		
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	1 (2%)
Adrenal gland, medulla	(48)	(46)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Pheochromocytoma benign		1 (2%)	1 (2%)
Islets, pancreatic	(49)	(50)	(49)
Adenoma	3 (6%)		
Parathyroid gland	(39)	(38)	(40)
Adenoma	1 (3%)		

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
ENDOCRINE SYSTEM (Continued)			
Pituitary gland	(49)	(49)	(48)
Pars distalis, adenoma	18 (37%)	18 (37%)	13 (27%)
Pars distalis, adenoma, multiple		1 (2%)	
Pars distalis, adenoma, two			1 (2%)
Pars distalis, carcinoma	2 (4%)		
Thyroid gland	(48)	(50)	(50)
Follicular cell, adenoma	2 (4%)	1 (2%)	3 (6%)
Follicular cell, adenoma, multiple		1 (2%)	
Follicular cell, adenoma, two	1 (2%)		
GENERAL BODY SYSTEM			
Tissue, NOS			(2)
Alveolar/bronchiolar carcinoma, metastatic, lung			2 (100%)
GENITAL SYSTEM			
Ovary	(48)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			2 (4%)
Luteoma		1 (2%)	
Uterus	(49)	(50)	(50)
Adenocarcinoma	1 (2%)		
Carcinoma		1 (2%)	
Hemangiosarcoma	2 (4%)	1 (2%)	1 (2%)
Polyp stromal	1 (2%)	3 (6%)	1 (2%)
HEMATOPOIETIC SYSTEM			
Bone marrow	(48)	(50)	(50)
Hemangioma		1 (2%)	
Hemangiosarcoma, metastatic, spleen			1 (2%)
Lymph node	(50)	(50)	(50)
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	1 (2%)
Lymph node, mandibular	(48)	(43)	(45)
Sarcoma, metastatic, skin			1 (2%)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Lymph node, mesenteric	(41)	(36)	(37)
Spleen	(48)	(49)	(50)
Hemangiosarcoma			4 (8%)
Thymus	(48)	(44)	(32)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (3%)
INTEGUMENTARY SYSTEM			
Mammary gland	(48)	(46)	(45)
Adenocarcinoma		1 (2%)	1 (2%)
Skin	(48)	(46)	(50)
Subcutaneous tissue, fibrosarcoma			1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)		
Subcutaneous tissue, hemangiosarcoma, metastatic, spleen			1 (2%)
Subcutaneous tissue, sarcoma			1 (2%)

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
MUSCULOSKELETAL SYSTEM			
Bone	(49)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, metastatic, lung			1 (2%)
Osteosarcoma	1 (2%)		
Skeletal muscle	(2)	(1)	
NERVOUS SYSTEM			
Brain	(49)	(50)	(50)
RESPIRATORY SYSTEM			
Larynx	(46)	(45)	(47)
Lung	(49)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	12 (24%)	10 (20%)
Alveolar/bronchiolar adenoma, multiple		7 (14%)	31 (62%)
Alveolar/bronchiolar carcinoma	3 (6%)	8 (16%)	5 (10%)
Alveolar/bronchiolar carcinoma, multiple		3 (6%)	40 (80%)
Hemangiosarcoma		1 (2%)	
Hepatocellular carcinoma, metastatic, liver	1 (2%)		
Osteosarcoma, metastatic, multiple, bone	1 (2%)		
Sarcoma, metastatic, skin			1 (2%)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung			2 (4%)
Nose	(49)	(50)	(50)
Mucosa, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Trachea	(50)	(50)	(50)
SPECIAL SENSES SYSTEM			
Harderian gland	(1)	(1)	(1)
Adenoma	1 (100%)	1 (100%)	
URINARY SYSTEM			
Kidney	(49)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			3 (6%)
Bilateral, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Ureter			(1)
Urinary bladder	(48)	(49)	(47)
SYSTEMIC LESIONS			
Multiple organs	*(50)	*(50)	*(50)
Lymphoma malignant histiocytic		1 (2%)	1 (2%)
Lymphoma malignant lymphocytic	3 (6%)	4 (8%)	3 (6%)
Lymphoma malignant mixed	7 (14%)	5 (10%)	8 (16%)
Lymphoma malignant undifferentiated cell	1 (2%)	3 (6%)	3 (6%)

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
TUMOR SUMMARY			
Total animals with primary neoplasms**	40	44	49
Total primary neoplasms	63	79	137
Total animals with benign neoplasms	29	36	45
Total benign neoplasms	38	49	65
Total animals with malignant neoplasms	22	24	47
Total malignant neoplasms	25	30	72
Total animals with secondary neoplasms***	2	1	11
Total secondary neoplasms	2	3	31

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: CHAMBER CONTROL

DAYS ON STUDY	214	382	480	544	563	590	603	605	606	613	619	619	627	629	642	642	655	657	704	729	729	729	729	729	729
CARCASS ID	03 1	50 1	05 1	09 1	11 1	26 1	48 1	44 1	24 1	43 1	39 1	46 1	20 1	49 1	30 1	32 1	36 1	18 1	37 1	01 1	02 1	04 1	33 1	40 1	41 1
ALIMENTARY SYSTEM																									
Esophagus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	+	+	+	A		+	+	+	+	+	A	+	+	M	+	+	+	+	+	+	+	+
Intestine large	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	–	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	M	M	+	+	+	+	–	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Intestine small	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	–	+	+	+	A	–	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	M	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+
Intestine small, jejunum	A	+	–	+	+	+	+	–	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+
Liver	A	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																	X			X				X	
Hepatocellular adenoma					X				X							X		X							
Hepatocellular adenoma, multiple							X																		
Mesentery																				+					
Pancreas		+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	M	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stomach	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	A	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	A	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth						+																			
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	–	+	+	+	+		+	+	+	+		+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	A	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Adrenal gland, cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal gland, medulla	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+
Islets, pancreatic		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X												
Parathyroid gland	M	+	+	+	M	+	+	+	+	M	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+
Adenoma				X																					
Pituitary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma						X										X	X		X	X			X		X
Pars distalis, carcinoma					X					X															
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																			X		X				
Follicular cell, adenoma, two																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Clitoral gland		+																							
Ovary	A	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Hemangiosarcoma																		X							
Polyp stromal																									X

+: Tissue examined microscopically
: Not examined
–: Present but not examined microscopically
I: Insufficient tissue

M: Missing
A: Autolysis precludes examination
X: Incidence of listed morphology

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: CHAMBER CONTROL (Continued)

DAYS ON STUDY	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	TOTAL:
CARCASS ID	421	451	471	061	071	081	101	121	131	141	151	161	171	191	211	221	231	251	271	281	291	311	341	351	381	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	42
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma					X																					4
Hepatocellular adenoma				X												X			X	X						8
Hepatocellular adenoma, multiple																										1
Mesentery															+			+								3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	M	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Tooth													+													2
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma															X											1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	48
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma													X								X					3
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	M	M	M	+	+	M	+	+	+	39
Adenoma																										1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma						X		X	X				X	X	X		X			X		X	X	X		18
Pars distalis, carcinoma																										2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell, adenoma																										2
Follicular cell, adenoma, two														X												1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Clitoral gland		+																								2
Ovary	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma																X										1
Hemangiosarcoma	X																									2
Polyp stromal																										1

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: CHAMBER CONTROL (Continued)

DAYS ON STUDY	214	382	480	544	563	590	603	605	606	613	619	619	627	629	642	642	655	657	704	729	729	729	729	729	729
CARCASS ID	03 1	50 1	05 1	09 1	11 1	26 1	48 1	44 1	24 1	43 1	39 1	46 1	20 1	49 1	30 1	32 1	36 1	18 1	37 1	01 1	02 1	04 1	33 1	40 1	41 1
HEMATOPOIETIC SYSTEM																									
Bone marrow	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	+	M	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	M	+	+	M	M
Spleen	M		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
INTEGUMENTARY SYSTEM																									
Mammary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Skin	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma																						X			
MUSCULOSKELETAL SYSTEM																									
Bone	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma														X											
Skeletal muscle												+			+										
NERVOUS SYSTEM																									
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Larynx	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+
Lung	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma											X														X
Hepatocellular carcinoma, metastatic, liver																				X					
Osteosarcoma, metastatic, multiple, bone														X											
Nose	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Harderian gland																									
Adenoma																									
URINARY SYSTEM																									
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic															X										
Lymphoma malignant mixed			X					X															X	X	
Lymphoma malignant undifferentiated cell type													X												

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: CHAMBER CONTROL (Continued)

DAYS ON STUDY	729	729	729	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	311	311	311	311	311	
CARCASS ID	42 1	45 1	47 1	06 1	07 1	08 1	10 1	12 1	13 1	14 1	15 1	16 1	17 1	19 1	21 1	22 1	23 1	25 1	27 1	28 1	29 1	31 1	34 1	35 1	38 1	TOTAL: TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	48
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	41
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	48
Subcutaneous tissue, hemangiosarcoma																										1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Osteosarcoma																										1
Skeletal muscle																										2
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
RESPIRATORY SYSTEM																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma																								X		1
Alveolar/bronchiolar carcinoma			X																							3
Hepatocellular carcinoma, metastatic, liver																										1
Osteosarcoma, metastatic, multiple, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Harderian gland		+																								1
Adenoma		X																								1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SYSTEMIC LESIONS																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic															X			X								3
Lymphoma malignant mixed				X	X			X																		7
Lymphoma malignant undifferentiated cell type																										1

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: 0.5 ppm

DAYS ON STUDY	1 2 8	2 6 3	3 3 5	3 8 9	5 2 4	5 7 6	5 9 2	6 0 1	6 0 1	6 1 2	6 6 5	6 6 6	6 9 2	6 9 2	6 9 3	6 9 9	7 0 1	7 0 3	7 0 6	7 1 5	7 1 6	7 1 8	7 3 0	7 3 0	7 3 0
CARCASS ID	2 0 6 1	2 5 0 1	2 0 7 1	2 3 7 1	2 2 6 1	2 2 1 1	2 3 5 1	2 0 4 1	2 1 6 1	2 3 8 1	2 1 5 1	2 0 8 1	2 3 9 1	2 4 2 1	2 0 9 1	2 3 1 1	2 4 9 1	2 1 0 1	2 1 8 1	2 3 6 1	2 0 5 1	2 2 0 1	2 0 1 1	2 0 2 1	2 0 3 1
ALIMENTARY SYSTEM																									
Esophagus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	+	+	+	+		+	+	A	M	+	+	A	+	M	+	+	A	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	M	M	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	M	+	A	+	+	+	+	+
Intestine large, rectum	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	A	M	+	+	A	-	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	M	+	+	+	+	+	+	A	+	+	+	+		M	+	+	M	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	A	+	+	+	A	-	+	+	+	A	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																						X			
Hepatocellular adenoma												+													
Mesentery																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																									
Stomach, glandular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARDIOVASCULAR SYSTEM																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung								X																	
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung								X																	
Adrenal gland, medulla	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+
Pheochromocytoma benign													X												
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	M	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	M
Pituitary gland	+	+	+	-	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X									X	X		X		X		X	X	
Pars distalis, adenoma, multiple																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																						X			
Follicular cell, adenoma, multiple																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Clitoral gland					+																				
Ovary	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Luteoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma				X																					
Hemangiosarcoma														X											
Polyp stromal											X												X		

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 0.5 ppm
(Continued)

DAYS ON STUDY	730	730	730	730	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	TOTAL:
CARCASS ID	21101	21201	21301	21401	21701	21901	22201	22301	22401	22501	22701	22801	22901	23301	24501	23001	23201	23401	24001	24101	24301	24401	24601	24701	24801	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+		40
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	44
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																			X							2
Hepatocellular adenoma																								X		1
Mesentery																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Papilloma squamous					X																					1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CARDIOVASCULAR SYSTEM																										
Blood vessel					+													+								2
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	M	+	M	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	M	+	+	+		38
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma		X		X		X	X		X				X					X		X		X	X	X		18
Pars distalis, adenoma, multiple			X																							1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
Follicular cell, adenoma, multiple																							X			1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Clitoral gland																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Luteoma																					X					1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Hemangiosarcoma																										1
Polyp stromal												X														3

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 0.5 ppm (Continued)

DAYS ON STUDY	128	263	335	389	524	576	592	601	601	612	665	666	692	692	693	699	701	703	706	715	716	718	730	730	730
CARCASS ID	20611	25011	20711	23711	22611	22111	23511	20411	21611	23811	21511	20811	23911	24211	20911	23111	24911	21011	21811	23611	20511	22011	20111	20211	20311
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung								X																	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	M	+
Lymph node, mesenteric	M	+	M	+	+	+	M	+	+	+	M	M	+	M	+	+	+	M	M	+	M	M	M	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	M	+
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+		+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	M	+	+	+	+
Adenocarcinoma																									
Skin	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MUSCULOSKELETAL SYSTEM																									
Bone																									
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Larynx	+	+	M	M	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X			X				X	X			X					X
Alveolar/bronchiolar adenoma, multiple																							X		
Alveolar/bronchiolar carcinoma																X									
Alveolar/bronchiolar carcinoma, multiple								X									X								
Hemangiosarcoma											X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Eye						+																			
Harderian gland																									
Adenoma																									
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																				X					
Lymphoma malignant lymphocytic												X			X								X		
Lymphoma malignant mixed					X								X			X						X			
Lymphoma malignant undifferentiated cell type		X																	X						

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 0.5 ppm (Continued)

DAYS ON STUDY	730	730	730	730	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	TOTAL:
CARCASS ID	21110	21121	21131	21141	21171	21191	22201	22231	22241	22251	22271	22281	22291	23311	24511	23011	23221	23411	24011	24111	24311	24411	24611	24711	24811	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																X										1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Lymph node, mandibular	+	+	+	+	+	+	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	43
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	36
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	44
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenocarcinoma																				X						1
Skin	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	M	46
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
RESPIRATORY SYSTEM																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			45
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X						X				X					X						X		X		12
Alveolar/bronchiolar adenoma, multiple						X			X	X					X					X	X					7
Alveolar/bronchiolar carcinoma				X										X			X			X			X	X	X	8
Alveolar/bronchiolar carcinoma, multiple																X										3
Hemangiosarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Eye							+																			2
Harderian gland							+																			1
Adenoma							X																			1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SYSTEMIC LESIONS																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic																				X						4
Lymphoma malignant mixed														X												5
Lymphoma malignant undifferentiated cell type																									X	3

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE: 2 ppm

DAYS ON STUDY	261	444	446	520	521	535	546	565	612	633	645	666	673	674	681	684	686	687	698	698	703	703	705	714
CARCASS ID	12001	13001	12201	12701	10801	10201	14901	14311	11711	14411	13211	13411	14711	13711	11611	14011	10511	14511	12811	13611	11211	11511	15011	12311	11101
ALIMENTARY SYSTEM																									
Esophagus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	+	+	A	A	A	A	+	A		+	+	+		A	+	A	+	+	+	+	M	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	M	+	A	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	M	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, ileum	A	M	+	+	A	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	M
Intestine small, jejunum	A	+	+	A	A	+	A	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hemangiosarcoma, metastatic, spleen																									
Hepatocellular carcinoma																									X
Hepatocellular adenoma											X														
Mesentery		+													+	+									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Tooth			+															+							
Peridontal tissue, alveolar/bronchiolar carcinoma, metastatic, lung			X																						
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung			X	X		X	X																		
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Extra adrenal tissue, alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Subcapsular, adenoma												X													
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																								X	
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung													X												
Pheochromocytoma benign																			X						
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+		+	M	+	M	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma														X		X			X	X					X
Pars distalis, adenoma, two																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
GENERAL BODY SYSTEM																									
Tissue, NOS			+	+																					
Alveolar/bronchiolar carcinoma, metastatic, lung			X	X																					
GENITAL SYSTEM																									
Clitoral gland																		+							
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung						X	X																		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																X									
Polyp stromal																									

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 2 ppm (Continued)

DAYS ON STUDY	726	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	TOTAL: TISSUES TUMORS
CARCASS ID	13111	10111	10311	10411	10611	10711	10911	11111	11311	11411	11811	11911	12111	12411	12511	12611	12911	13311	13511	13811	13911	14111	14211	14611	14811	
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	M	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	36
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																			X							1
Hemangiosarcoma, metastatic, spleen		X					X																			2
Hepatocellular carcinoma										X															X	3
Hepatocellular adenoma							X						X					X								4
Mesentery													+											+		5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth																										2
Peridontal tissue, alveolar/bronchiolar carcinoma, metastatic, lung																										1
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung				X																						5
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Extra adrenal tissue, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Subcapsular, adenoma																										1
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	M	+	+	+	+	+	+	47
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	M	+	+	+	+	+	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma			X	X		X				X			X			X					X	X				13
Pars distalis, adenoma, two																		X								1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma				X														X							X	3
GENERAL BODY SYSTEM																										
Tissue, NOS																										2
Alveolar/bronchiolar carcinoma, metastatic, lung																										2
GENITAL SYSTEM																										
Clitoral gland																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Polyp stromal		X																								1

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 2 ppm (Continued)

DAYS ON STUDY	261	444	446	520	521	535	546	565	612	633	645	666	673	674	681	684	686	687	698	698	703	703	703	705	714
CARCASS ID	12001	13001	12201	12701	10801	10201	14901	14301	11701	14401	13201	13401	14701	13701	11601	14001	10501	14501	12801	13601	11201	11501	15001	12301	11101
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, spleen																									
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung							X																		
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung						X																			
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Sarcoma, metastatic, skin													X												
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Lymph node, mesenteric	M	+	+		+	+	+	+	M	+	+	+	M	M	+	+	+	M	+	M	M	M	M	+	M
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma												X													
Thymus		+	M	M	+	+	+	M	+	M	M	+	M	+	+	M	+	A	+	+	+	M	+	M	M
Alveolar/bronchiolar carcinoma, metastatic, lung																									
INTEGUMENTARY SYSTEM																									
Mammary gland	M	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	M	+	+	+		+	+	+	+
Adenocarcinoma																								X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma																		X							
Subcutaneous tissue, hemangiosarcoma, metastatic, spleen																									
Subcutaneous tissue, sarcoma													X												
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, metastatic, lung						X																			
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Larynx	+	+	+		+	+	+	M	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X										X		X		X		
Alveolar/bronchiolar adenoma, multiple		X		X	X	X	X	X		X				X	X	X	X	X		X		X			X
Alveolar/bronchiolar carcinoma					X	X					X								X					X	
Alveolar/bronchiolar carcinoma, multiple		X	X	X			X	X		X		X	X	X			X	X		X	X	X	X		X
Sarcoma, metastatic, skin													X												
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung							X				X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mucosa, alveolar/bronchiolar carcinoma, metastatic, lung			X																						
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Harderian gland					+																				
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X			X																		
Bilateral, alveolar/bronchiolar carcinoma, metastatic, lung			X																						
Ureter		+																							
Urinary bladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M
SYSTEMIC LESIONS																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic														X											
Lymphoma malignant lymphocytic								X																	
Lymphoma malignant mixed					X					X	X					X	X	X							
Lymphoma malignant undifferentiated cell type		X																							

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 2 ppm (Continued)

DAYS ON STUDY	726	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	TOTAL:
CARCASS ID	13111	10111	10311	10441	10061	10071	10091	11111	11131	11141	11181	11191	12111	12441	12551	12661	12991	13331	13551	13881	13991	14111	14421	14661	14881	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, spleen							X																			1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Sarcoma, metastatic, skin																										1
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Lymph node, mesenteric	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	37
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma		X					X											X								4
Thymus	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	M	+	M	+	+	+	M	M	+	32
Alveolar/bronchiolar carcinoma, metastatic, lung				X																						1
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Adenocarcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, hemangiosarcoma, metastatic, spleen		X																								1
Subcutaneous tissue, sarcoma																										1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, metastatic, lung																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
RESPIRATORY SYSTEM																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X					X		X											X	X	X					10
Alveolar/bronchiolar adenoma, multiple				X			X		X	X	X	X	X	X	X	X	X	X				X	X	X	X	31
Alveolar/bronchiolar carcinoma																										5
Alveolar/bronchiolar carcinoma, multiple	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	40
Sarcoma, metastatic, skin																										1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mucosa, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Harderian gland																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung			X																							3
Bilateral, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Ureter																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
SYSTEMIC LESIONS																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic		X															X									3
Lymphoma malignant mixed																X		X								8
Lymphoma malignant undifferentiated cell type									X															X		3

TABLE D3. ANALYSIS OF PRIMARY NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	0.5 ppm	2 ppm
Pancreatic Islets: Adenoma			
Overall Rates (a)	3/49 (6%)	0/50 (0%)	0/49 (0%)
Adjusted Rates (b)	8.9%	0.0%	0.0%
Terminal Rates (c)	2/31 (6%)	0/28 (0%)	0/24 (0%)
Day of First Observation	627		
Life Table Tests (d)	P=0.133N	P=0.132N	P=0.149N
Logistic Regression Tests (d)	P=0.117N	P=0.119N	P=0.123N
Cochran-Armitage Trend Test (d)	P=0.115N		
Fisher Exact Test (d)		P=0.117N	P=0.121N
Liver: Hepatocellular Adenoma			
Overall Rates (a)	9/49 (18%)	1/50 (2%)	4/50 (8%)
Adjusted Rates (b)	23.4%	3.6%	14.7%
Terminal Rates (c)	4/31 (13%)	1/28 (4%)	3/24 (13%)
Day of First Observation	563	729	645
Life Table Tests (d)	P=0.277N	P=0.014N	P=0.174N
Logistic Regression Tests (d)	P=0.207N	P=0.009N	P=0.110N
Cochran-Armitage Trend Test (d)	P=0.205N		
Fisher Exact Test (d)		P=0.007N	P=0.109N
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	4/49 (8%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	12.3%	7.1%	11.9%
Terminal Rates (c)	3/31 (10%)	2/28 (7%)	2/24 (8%)
Day of First Observation	655	729	714
Life Table Tests (d)	P=0.600	P=0.364N	P=0.601N
Logistic Regression Tests (d)	P=0.558N	P=0.315N	P=0.505N
Cochran-Armitage Trend Test (d)	P=0.532N		
Fisher Exact Test (d)		P=0.329N	P=0.489N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	13/49 (27%)	3/50 (6%)	7/50 (14%)
Adjusted Rates (b)	33.9%	10.7%	25.8%
Terminal Rates (c)	7/31 (23%)	3/28 (11%)	5/24 (21%)
Day of First Observation	563	729	645
Life Table Tests (d)	P=0.329N	P=0.011N	P=0.193N
Logistic Regression Tests (d)	P=0.216N	P=0.007N	P=0.102N
Cochran-Armitage Trend Test (d)	P=0.208N		
Fisher Exact Test (d)		P=0.005N	P=0.096N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	1/49 (2%)	19/50 (38%)	41/50 (82%)
Adjusted Rates (b)	3.2%	56.6%	93.0%
Terminal Rates (c)	1/31 (3%)	14/28 (50%)	21/24 (88%)
Day of First Observation	729	601	444
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Logistic Regression Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P<0.001	P<0.001
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	3/49 (6%)	11/50 (22%)	45/50 (90%)
Adjusted Rates (b)	8.8%	34.2%	97.8%
Terminal Rates (c)	2/31 (6%)	8/28 (29%)	23/24 (96%)
Day of First Observation	619	601	444
Life Table Tests (d)	P<0.001	P=0.017	P<0.001
Logistic Regression Tests (d)	P<0.001	P=0.023	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.022	P<0.001

TABLE D3. ANALYSIS OF PRIMARY NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	4/49 (8%)	24/50 (48%)	49/50 (98%)
Adjusted Rates (b)	11.9%	69.7%	100.0%
Terminal Rates (c)	3/31 (10%)	18/28 (64%)	24/24 (100%)
Day of First Observation	619	601	444
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Logistic Regression Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P<0.001	P<0.001
Pituitary Gland/Pars Distalis: Adenoma			
Overall Rates (a)	18/49 (37%)	19/49 (39%)	14/48 (29%)
Adjusted Rates (b)	51.0%	56.9%	48.1%
Terminal Rates (c)	14/31 (45%)	14/28 (50%)	9/23 (39%)
Day of First Observation	590	592	674
Life Table Tests (d)	P=0.484N	P=0.401	P=0.532N
Logistic Regression Tests (d)	P=0.219N	P=0.543	P=0.278N
Cochran-Armitage Trend Test (d)	P=0.216N		
Fisher Exact Test (d)		P=0.500	P=0.282N
Pituitary Gland/Pars Distalis: Adenoma or Carcinoma			
Overall Rates (a)	20/49 (41%)	19/49 (39%)	14/48 (29%)
Adjusted Rates (b)	53.2%	56.9%	48.1%
Terminal Rates (c)	14/31 (45%)	14/28 (50%)	9/23 (39%)
Day of First Observation	563	592	674
Life Table Tests (d)	P=0.356N	P=0.559	P=0.373N
Logistic Regression Tests (d)	P=0.132N	P=0.494N	P=0.159N
Cochran-Armitage Trend Test (d)	P=0.132N		
Fisher Exac. Test (d)		P=0.500N	P=0.161N
Thyroid Gland: Follicular Cell Adenoma			
Overall Rates (a)	3/48 (6%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	9.6%	6.9%	12.5%
Terminal Rates (c)	2/30 (7%)	1/28 (4%)	3/24 (13%)
Day of First Observation	704	718	729
Life Table Tests (d)	P=0.468	P=0.525N	P=0.561
Logistic Regression Tests (d)	P=0.546	P=0.462N	P=0.650
Cochran-Armitage Trend Test (d)	P=0.586		
Fisher Exact Test (d)		P=0.480N	P=0.641N
Uterus: Stromal Polyp			
Overall Rates (e)	1/49 (2%)	3/50 (6%)	1/50 (2%)
Adjusted Rates (b)	3.2%	9.5%	4.2%
Terminal Rates (c)	1/31 (3%)	2/28 (7%)	1/24 (4%)
Day of First Observation	729	665	729
Life Table Tests (d)	P=0.582N	P=0.298	P=0.704
Logistic Regression Tests (d)	P=0.527N	P=0.317	P=0.704
Cochran-Armitage Trend Test (d)	P=0.512N		
Fisher Exact Test (d)		P=0.316	P=0.747N
Circulatory System: Hemangiosarcoma			
Overall Rates (e)	3/50 (6%)	2/50 (4%)	6/50 (12%)
Adjusted Rates (b)	9.3%	5.1%	21.1%
Terminal Rates (c)	2/31 (6%)	0/28 (0%)	4/24 (17%)
Day of First Observation	657	665	666
Life Table Tests (d)	P=0.101	P=0.466N	P=0.190
Logistic Regression Tests (d)	P=0.125	P=0.498N	P=0.239
Cochran-Armitage Trend Test (d)	P=0.128		
Fisher Exact Test (d)		P=0.500N	P=0.243

TABLE D3. ANALYSIS OF PRIMARY NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
Circulatory System: Hemangioma or Hemangiosarcoma			
Overall Rates (e)	3/50 (6%)	3/50 (6%)	6/50 (12%)
Adjusted Rates (b)	9.3%	8.5%	21.1%
Terminal Rates (c)	2/31 (6%)	1/28 (4%)	4/24 (17%)
Day of First Observation	657	665	666
Life Table Tests (d)	P=0.127	P=0.639N	P=0.190
Logistic Regression Tests (d)	P=0.160	P=0.659N	P=0.239
Cochran-Armitage Trend Test (d)	P=0.165		
Fisher Exact Test (d)		P=0.661N	P=0.243
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (e)	11/50 (22%)	13/50 (26%)	15/50 (30%)
Adjusted Rates (b)	29.9%	33.4%	40.6%
Terminal Rates (c)	7/31 (23%)	4/28 (14%)	6/24 (25%)
Day of First Observation	480	263	444
Life Table Tests (d)	P=0.168	P=0.394	P=0.185
Logistic Regression Tests (d)	P=0.237	P=0.408	P=0.247
Cochran-Armitage Trend Test (d)	P=0.238		
Fisher Exact Test (d)		P=0.408	P=0.247
All Sites: Benign Tumors			
Overall Rates (e)	29/50 (58%)	36/50 (72%)	45/50 (90%)
Adjusted Rates (b)	70.2%	92.2%	97.8%
Terminal Rates (c)	19/31 (61%)	25/28 (89%)	23/24 (96%)
Day of First Observation	554	592	444
Life Table Tests (d)	P<0.001	P=0.086	P<0.001
Logistic Regression Tests (d)	P<0.001	P=0.093	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.104	P<0.001
All Sites: Malignant Tumors			
Overall Rates (e)	22/50 (44%)	24/50 (48%)	47/50 (94%)
Adjusted Rates (b)	52.7%	56.0%	100.0%
Terminal Rates (c)	12/31 (39%)	10/28 (36%)	24/24 (100%)
Day of First Observation	480	263	444
Life Table Tests (d)	P<0.001	P=0.408	P<0.001
Logistic Regression Tests (d)	P<0.001	P=0.420	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Test (d)		P=0.421	P<0.001
All Sites: All Tumors			
Overall Rates (e)	40/50 (80%)	44/50 (88%)	49/50 (98%)
Adjusted Rates (b)	85.0%	97.7%	100.0%
Terminal Rates (c)	24/31 (77%)	27/28 (96%)	24/24 (100%)
Day of First Observation	480	263	444
Life Table Tests (d)	P=0.012	P=0.216	P=0.022
Logistic Regression Tests (d)	P=0.004	P=0.162	P=0.004
Cochran-Armitage Trend Test (d)	P=0.005		
Fisher Exact Test (d)		P=0.207	P=0.004

(a) Number of tumor-bearing animals/number of animals examined microscopically at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence in animals killed at the end of the study
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors 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. A negative trend or lower incidence in a dosed group than in controls is indicated by (N).
(e) Number of tumor-bearing animals/number of animals examined grossly at the site

TABLE D4. HISTORICAL INCIDENCE OF ALVEOLAR/BRONCHIOLAR NEOPLASMS IN FEMALE $B6C3F_1$ MICE (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence for Chamber Controls in NTP Studies (b)			
Propylene oxide	4/50	0/50	4/50
Methyl methacrylate	1/49	1/49	2/49
Propylene	6/50	0/50	6/50
1,2-Epoxybutane	2/50	2/50	4/50
Dichloromethane	2/50	1/50	3/50
Ethylene oxide	2/49	0/49	2/49
Bromoethane	3/50	3/50	6/50
Tetrachloroethylene	4/48	2/48	6/48
TOTAL	24/396 (6.1%)	9/396 (2.3%)	33/396 (8.3%)
SD (c)	3.22%	2.27%	3.51%
Range (d)			
High	6/50	3/50	6/48
Low	1/49	0/50	2/49
Overall Historical Incidence for Untreated Controls in NTP Studies			
TOTAL	73/1,676 (4.4%)	35/1,676 (2.1%)	107/1,676 (6.4%)
SD (c)	3.35%	1.68%	3.76%
Range (d)			
High	6/49	3/50	8/50
Low	0/50	0/50	0/50

(a) Data as of March 1, 1989, for studies of at least 104 weeks
(b) All inhalation studies included in the NTP historical data base were conducted at Battelle Pacific Northwest Laboratories.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE

	Chamber Control	0.5 ppm	2 ppm
DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Early deaths			
Natural death	5	4	10
Accidentally killed	2	1	
Moribund sacrifice	12	17	16
Survivors			
Terminal sacrifice	30	27	23
Natural death	1		
Moribund sacrifice		1	1
Animals examined microscopically	50	50	50
ALIMENTARY SYSTEM			
Gallbladder	(42)	(40)	(36)
Infiltration cellular, lymphocytic, multifocal		1 (3%)	
Intestine large, cecum	(47)	(44)	(45)
Peyer's patch, hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)
Intestine large, colon	(49)	(47)	(46)
Infiltration cellular, lymphocytic, multifocal		1 (2%)	
Intestine small, jejunum	(45)	(46)	(44)
Inflammation, granulomatous, focal	1 (2%)		
Liver	(49)	(50)	(50)
Basophilic focus	1 (2%)		1 (2%)
Cytomegaly		1 (2%)	
Eosinophilic focus	3 (6%)		
Fatty change, focal		1 (2%)	
Granuloma, multifocal	1 (2%)		1 (2%)
Hematopoietic cell proliferation, multifocal	1 (2%)	10 (20%)	7 (14%)
Infiltration cellular, lymphocytic, focal		1 (2%)	
Infiltration cellular, lymphocytic, multifocal	4 (8%)	2 (4%)	6 (12%)
Inflammation, acute, multifocal		1 (2%)	
Inflammation, subacute, multifocal	4 (8%)	3 (6%)	
Necrosis, acute, multifocal	3 (6%)	4 (8%)	3 (6%)
Necrosis, chronic, multifocal		1 (2%)	
Necrosis, subacute, focal	2 (4%)	2 (4%)	
Vacuolization cytoplasmic, focal	1 (2%)		
Vacuolization cytoplasmic, multifocal		1 (2%)	
Bile duct, hyperplasia, multifocal			1 (2%)
Centrilobular, fatty change, diffuse			1 (2%)
Centrilobular, necrosis, acute, diffuse			1 (2%)
Periportal, vacuolization cytoplasmic, diffuse		1 (2%)	
Portal, inflammation, chronic, multifocal		2 (4%)	
Right lateral lobe, hepatodiaphragmatic nodule	1 (2%)		1 (2%)
Vein, thrombus			1 (2%)
Mesentery	(3)	(1)	(5)
Infiltration cellular, lymphocytic, multifocal	3 (100%)		1 (20%)
Inflammation, suppurative			1 (20%)
Pancreas	(49)	(50)	(50)
Abscess, multiple			1 (2%)
Cyst		1 (2%)	
Infiltration cellular, lymphocytic, multifocal	10 (20%)	9 (18%)	7 (14%)
Acinus, atrophy, diffuse		1 (2%)	1 (2%)
Acinus, atrophy, multifocal	1 (2%)		
Salivary glands	(48)	(50)	(50)
Infiltration cellular, lymphocytic, focal		1 (2%)	1 (2%)
Infiltration cellular, lymphocytic, multifocal	26 (54%)	17 (34%)	19 (38%)
Stomach, forestomach	(48)	(50)	(49)
Abscess		1 (2%)	
Epithelium, hyperplasia, diffuse		1 (2%)	
Epithelium, hyperplasia, focal	2 (4%)		
Epithelium, hyperplasia, multifocal	1 (2%)		

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
ALIMENTARY SYSTEM (Continued)			
Stomach, glandular	(47)	(49)	(49)
Infiltration cellular, lymphocytic, focal		1 (2%)	
Infiltration cellular, lymphocytic, multifocal	1 (2%)	1 (2%)	
Necrosis, acute, multifocal		1 (2%)	
Mucosa, degeneration, multifocal		1 (2%)	
Tooth	(2)		(2)
Abscess	1 (50%)		
Developmental malformation			1 (50%)
Inflammation, chronic active	1 (50%)		
CARDIOVASCULAR SYSTEM			
Blood vessel		(2)	
Aorta, inflammation, chronic active, focal		1 (50%)	
Artery, inflammation, chronic, multifocal		1 (50%)	
Heart	(47)	(50)	(50)
Cardiomyopathy	1 (2%)		
Infiltration cellular, lymphocytic, multifocal	1 (2%)	1 (2%)	
Inflammation, acute, multifocal	1 (2%)		
Inflammation, chronic, multifocal		1 (2%)	
Mineralization, multifocal		1 (2%)	
Necrosis, acute, focal		1 (2%)	
Pigmentation			1 (2%)
Artery, mineralization, multifocal			1 (2%)
Atrium left, thrombus		1 (2%)	1 (2%)
Epicardium, inflammation, chronic, focal			1 (2%)
Epicardium, inflammation, chronic active, focal	1 (2%)		1 (2%)
Epicardium, inflammation, suppurative			1 (2%)
Mitral valve, bacterium	2 (4%)		
Mitral valve, inflammation, chronic active	2 (4%)		
Valve, pigmentation			1 (2%)
ENDOCRINE SYSTEM			
Adrenal gland	(49)	(49)	(50)
Capsule, accessory adrenal cortical nodule	1 (2%)	2 (4%)	1 (2%)
Capsule, accessory adrenal cortical nodule, multiple	1 (2%)		
Capsule, inflammation, suppurative, chronic active			1 (2%)
Subcapsular, hyperplasia, diffuse	4 (8%)	4 (8%)	4 (8%)
Subcapsular, hyperplasia, focal	1 (2%)		1 (2%)
Subcapsular, hyperplasia, multifocal	43 (88%)	43 (88%)	43 (86%)
Adrenal gland, cortex	(49)	(48)	(50)
Cyst		1 (2%)	
Hemorrhage, acute			1 (2%)
Hyperplasia, focal		1 (2%)	1 (2%)
Hyperplasia, multifocal	1 (2%)		
Adrenal gland, medulla	(48)	(46)	(47)
Hyperplasia, focal	2 (4%)		2 (4%)
Islets, pancreatic	(49)	(50)	(49)
Hyperplasia, focal		3 (6%)	3 (6%)
Hyperplasia, multifocal	10 (20%)	2 (4%)	3 (6%)
Hypoplasia		1 (2%)	
Pituitary gland	(49)	(49)	(48)
Congestion			1 (2%)
Cyst	2 (4%)		1 (2%)
Pars distalis, angiectasis	8 (16%)	7 (14%)	3 (6%)
Pars distalis, hyperplasia	1 (2%)		

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
ENDOCRINE SYSTEM			
Pituitary gland (Continued)	(49)	(49)	(48)
Pars distalis, hyperplasia, focal	4 (8%)	4 (8%)	4 (8%)
Pars distalis, hyperplasia, multifocal		2 (4%)	
Pars distalis, hypertrophy, focal	5 (10%)	1 (2%)	
Pars distalis, hypertrophy, multifocal	1 (2%)		
Thyroid gland	(48)	(50)	(50)
Cyst		2 (4%)	1 (2%)
Infiltration cellular, lymphocytic, focal	1 (2%)	2 (4%)	
Infiltration cellular, lymphocytic, multifocal	3 (6%)	1 (2%)	
Inflammation, acute, focal	1 (2%)	1 (2%)	
Inflammation, chronic, focal		1 (2%)	2 (4%)
Inflammation, chronic, multifocal	4 (8%)	2 (4%)	2 (4%)
Inflammation, chronic active, focal	1 (2%)	2 (4%)	1 (2%)
Inflammation, chronic active, multifocal	2 (4%)	2 (4%)	
C-cell, hyperplasia, focal	1 (2%)		
Follicle, cyst		1 (2%)	
Follicle, cyst, multiple	1 (2%)	2 (4%)	3 (6%)
Follicular cell, hyperplasia, diffuse	1 (2%)		
Follicular cell, hyperplasia, focal	1 (2%)	3 (6%)	2 (4%)
Follicular cell, hyperplasia, multifocal	7 (15%)	9 (18%)	5 (10%)
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Clitoral gland	(2)	(1)	(1)
Atrophy	1 (50%)		
Cyst			1 (100%)
Cyst, multiple	1 (50%)	1 (100%)	
Ovary	(48)	(49)	(50)
Abscess			1 (2%)
Angiectasis, focal			1 (2%)
Cyst	8 (17%)	8 (16%)	9 (18%)
Metaplasia, osseous, focal			1 (2%)
Mineralization		1 (2%)	
Pigmentation		1 (2%)	1 (2%)
Thrombus	1 (2%)		
Interstitium, hyperplasia	1 (2%)		
Periovarian tissue, infiltration cellular, lymphocytic	3 (6%)	1 (2%)	2 (4%)
Periovarian tissue, inflammation, chronic active			1 (2%)
Uterus	(49)	(50)	(50)
Abscess			1 (2%)
Abscess, multiple			1 (2%)
Adenomyosis	1 (2%)		
Amyloid deposition	1 (2%)		
Angiectasis, multifocal	1 (2%)		
Cyst		1 (2%)	3 (6%)
Dilatation		2 (4%)	1 (2%)
Hyperplasia, atypical, glandular, focal		1 (2%)	
Hyperplasia, cystic	1 (2%)	3 (6%)	3 (6%)
Infiltration cellular, lymphocytic, multifocal	1 (2%)		
Inflammation, chronic active			1 (2%)
Thrombus	1 (2%)		
Thrombus, multiple			1 (2%)
Bilateral, hyperplasia, cystic	33 (67%)	40 (80%)	35 (70%)
Epithelium, hyperplasia, focal			1 (2%)

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
HEMATOPOIETIC SYSTEM			
Bone marrow	(48)	(50)	(50)
Atrophy, diffuse		1 (2%)	
Atrophy, focal			1 (2%)
Myeloid cell, hyperplasia		1 (2%)	3 (6%)
Lymph node	(50)	(50)	(50)
Hemorrhage, acute			1 (2%)
Inguinal, hyperplasia, lymphoid	2 (4%)	3 (6%)	
Lumbar, hyperplasia, lymphoid			1 (2%)
Mediastinal, hyperplasia		1 (2%)	1 (2%)
Mediastinal, hyperplasia, lymphoid	1 (2%)	1 (2%)	4 (8%)
Pancreatic, hemorrhage, acute			1 (2%)
Renal, hematopoietic cell proliferation		1 (2%)	
Renal, hemorrhage, subacute	1 (2%)		
Renal, hyperplasia, lymphoid	1 (2%)		
Lymph node, mandibular	(48)	(43)	(45)
Cyst		3 (7%)	
Hemorrhage, acute		2 (5%)	
Hemorrhage, subacute	1 (2%)		1 (2%)
Hyperplasia	1 (2%)		1 (2%)
Hyperplasia, lymphoid	6 (13%)	6 (14%)	7 (16%)
Hyperplasia, re cell			1 (2%)
Infiltration cellular, histiocytic			1 (2%)
Inflammation, subacute			1 (2%)
Pigmentation, hemosiderin	1 (2%)		2 (4%)
Lymph node, mesenteric	(41)	(36)	(37)
Angiectasis	1 (2%)		
Depletion lymphoid	1 (2%)	1 (3%)	
Hematopoietic cell proliferation	4 (10%)	4 (11%)	5 (14%)
Hemorrhage, acute	10 (24%)	4 (11%)	9 (24%)
Hemorrhage, subacute	2 (5%)	1 (3%)	
Hyperplasia, lymphoid	1 (2%)	3 (8%)	
Spleen	(48)	(49)	(50)
Ectasia, focal			1 (2%)
Edema	1 (2%)		
Hematopoietic cell proliferation	10 (21%)	9 (18%)	14 (28%)
Hyperplasia, lymphoid	2 (4%)	5 (10%)	5 (10%)
Infarct	1 (2%)		
Pigmentation, hemosiderin	1 (2%)	2 (4%)	
Capsule, ectopic tissue		1 (2%)	
Capsule, inflammation, chronic active			1 (2%)
Thymus	(48)	(44)	(32)
Angiectasis, focal	1 (2%)		
Depletion lymphoid	4 (8%)	6 (14%)	6 (19%)
Ectopic parathyroid gland	3 (6%)	1 (2%)	2 (6%)
Hyperplasia, lymphoid	3 (6%)	6 (14%)	4 (13%)
Metaplasia, osseous, focal			1 (3%)
INTEGUMENTARY SYSTEM			
Mammary gland	(48)	(46)	(45)
Ectasia, multifocal	8 (17%)	4 (9%)	2 (4%)
Hyperplasia, diffuse	7 (15%)	8 (17%)	6 (13%)
Inflammation, chronic active, multifocal	1 (2%)		
Skin	(48)	(46)	(50)
Inflammation, chronic active, focal	1 (2%)		
Inflammation, chronic active, multifocal	1 (2%)		
Hair follicle, inflammation, chronic active		1 (2%)	
Subcutaneous tissue, edema		1 (2%)	
Subcutaneous tissue, inflammation, chronic active			1 (2%)

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
MUSCULOSKELETAL SYSTEM			
Bone	(49)	(50)	(50)
Fibrous osteodystrophy	12 (24%)	10 (20%)	10 (20%)
Osteoporosis, focal		1 (2%)	
Skeletal muscle	(2)	(1)	
Inflammation, chronic, focal	1 (50%)		
NERVOUS SYSTEM			
Brain	(49)	(50)	(50)
Abscess			1 (2%)
Compression	9 (18%)	2 (4%)	8 (16%)
Hemorrhage, multifocal	1 (2%)		
Hydrocephalus	2 (4%)		1 (2%)
Mineralization, multifocal	26 (53%)	26 (52%)	20 (40%)
Meninges, infiltration cellular, lymphocytic, multifocal			1 (2%)
Perivascular, infiltration cellular, lymphocytic, multifocal	2 (4%)		3 (6%)
RESPIRATORY SYSTEM			
Larynx	(46)	(45)	(47)
Exudate			1 (2%)
Infiltration cellular, lymphocytic, multifocal	2 (4%)		1 (2%)
Mineralization			1 (2%)
Lung	(49)	(50)	(50)
Embolus tumor, multiple			1 (2%)
Hemorrhage, acute, focal			1 (2%)
Hemorrhage, acute, multifocal		1 (2%)	
Hemorrhage, subacute, multifocal		1 (2%)	
Infiltration cellular, lymphocytic, diffuse		1 (2%)	
Infiltration cellular, lymphocytic, focal	1 (2%)		
Infiltration cellular, lymphocytic, multifocal	16 (33%)	2 (4%)	2 (4%)
Inflammation, acute, focal		1 (2%)	
Pigmentation, hemosiderin, diffuse		1 (2%)	
Alveolar epithelium, hyperplasia, focal	2 (4%)	13 (26%)	
Alveolar epithelium, hyperplasia, multifocal		7 (14%)	41 (82%)
Alveolus, infiltration cellular, histiocytic, diffuse	1 (2%)	2 (4%)	
Alveolus, infiltration cellular, histiocytic, focal	1 (2%)		
Alveolus, infiltration cellular, histiocytic, multifocal	1 (2%)	8 (16%)	32 (64%)
Bronchiole, hyperplasia, focal		2 (4%)	
Bronchiole, hyperplasia, multifocal		5 (10%)	41 (82%)
Interstitium, inflammation, chronic active, focal		1 (2%)	
Mediastinum, infiltration cellular, lymphocytic, multifocal			2 (4%)
Mediastinum, inflammation, chronic			1 (2%)
Pleura, hyperplasia			1 (2%)
Pleura, infiltration cellular, lymphocytic		2 (4%)	
Nose	(49)	(50)	(50)
Lumen, exudate	3 (6%)	30 (60%)	33 (66%)
Lumen, foreign body		1 (2%)	
Mucosa, inflammation, chronic	2 (4%)	1 (2%)	
Mucosa, inflammation, chronic active	9 (18%)	10 (20%)	23 (46%)
Mucosa, ulcer	1 (2%)	2 (4%)	
Nasolacrimal duct, exudate		1 (2%)	
Nasolacrimal duct, inflammation, chronic		1 (2%)	

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF TETRANITROMETHANE (Continued)

	Chamber Control	0.5 ppm	2 ppm
RESPIRATORY SYSTEM			
Nose (Continued)	(49)	(50)	(50)
Nasolacrimal duct, inflammation, chronic active	1 (2%)	1 (2%)	
Olfactory epithelium, atrophy	2 (4%)	4 (8%)	2 (4%)
Respiratory epithelium, hyperplasia	2 (4%)	5 (10%)	17 (34%)
Respiratory epithelium, metaplasia, squamous		2 (4%)	8 (16%)
Sinus, hyperplasia			1 (2%)
Sinus, inflammation, chronic active			1 (2%)
Trachea	(50)	(50)	(50)
Inflammation, chronic	2 (4%)	2 (4%)	
Inflammation, chronic active	2 (4%)		
SPECIAL SENSES SYSTEM			
Eye		(2)	
Abscess		1 (50%)	
Atrophy		1 (50%)	
Harderian gland	(1)	(1)	(1)
Inflammation, chronic active		1 (100%)	
URINARY SYSTEM			
Kidney	(49)	(50)	(50)
Cytoplasmic alteration, multifocal	1 (2%)		
Infarct		3 (6%)	
Infiltration cellular, lymphocytic	20 (41%)	14 (28%)	15 (30%)
Inflammation, acute, multifocal	1 (2%)		
Metaplasia, osseous, focal	1 (2%)		1 (2%)
Nephropathy, chronic		2 (4%)	
Pigmentation, diffuse			1 (2%)
Cortex, mineralization, multifocal			1 (2%)
Interstitial tissue, inflammation, chronic, multifocal			2 (4%)
Renal tubule, hyperplasia, focal			1 (2%)
Renal tubule, necrosis, acute, multifocal			1 (2%)
Urinary bladder	(48)	(49)	(47)
Infiltration cellular, lymphocytic, focal	3 (6%)		1 (2%)
Infiltration cellular, lymphocytic, multifocal	18 (38%)	17 (35%)	17 (36%)
Artery, inflammation, chronic active, multifocal			1 (2%)

APPENDIX E

RESULTS OF SEROLOGIC ANALYSIS

APPENDIX E. RESULTS OF SEROLOGIC ANALYSIS

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.

Blood was collected from six control, two low dose, and two high dose male mice B6C3F$_1$ mice killed at 12 months. Data from animals surviving 24 months were collected from 5/50 randomly selected control animals of each sex and species. The blood from each animal was collected and clotted, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the viral antibody titers. The following tests were performed:

	Hemagglutination Inhibition	Complement Fixation	ELISA
Mice	PVM (pneumonia virus of mice) Reo 3 (reovirus type 3) GDVII (Theiler's encephalomyelitis virus) Poly (polyoma virus) MVM (minute virus of mice) Sendai	M. Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus	MHV (mouse hepatitis virus) (12, 24 mo) Ectro (infectious ectromelia)
Rats	Sendai KRV (Kilham rat virus) H-1 (Toolan's H-1 virus)		RCV (rat coronavirus) PVM

Results

Results are presented in Table E1.

TABLE E1. MURINE VIRUS ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR INHALATION STUDIES OF TETRANITROMETHANE (a)

Interval (months)	Number of Animals	Positive Serologic Reaction for
RATS		
24	--	None positive
MICE		
12	--	None positive
24	7/10	MVM

(a) Blood samples were taken from six control, two low dose, and two high dose male mice when they were killed 12 months after the start of dosing and from the control animals just before they were killed at the end of the studies at 24 months; samples were sent to Microbiological Associates, Inc. (Bethesda, MD) for determination of antibody titers.

APPENDIX F

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

Pellet Diet: March 1982 to March 1982

(Manufactured by Zeigler Bros., Inc., Gardners, PA)

TABLE F1. INGREDIENTS OF NIH 07 RAT AND MOUSE RATION (a)

Ingredients (b)	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

(a) NCI, 1976; NIH, 1978
(b) Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE F2. VITAMINS AND MINERALS IN NIH 07 RAT AND MOUSE RATION (a)

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 µg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

(a) Per ton (2,000 lb) of finished product

TABLE F3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Protein (percent by weight)	23.26 ± 1.04	21.3-26.3	26
Crude fat (percent by weight)	5.07 ± 0.55	3.3-5.7	26
Crude fiber (percent by weight)	3.44 ± 0.51	2.9-5.6	26
Ash (percent by weight)	6.56 ± 0.42	5.7-7.3	26
Amino Acids (percent of total diet)			
Arginine	1.320 ± 0.072	1.310-1.390	5
Cystine	0.319 ± 0.088	0.218-0.400	5
Glycine	1.146 ± 0.063	1.060-1.210	5
Histidine	0.571 ± 0.026	0.531-0.603	5
Isoleucine	0.914 ± 0.030	0.881-0.944	5
Leucine	1.946 ± 0.056	1.850-1.990	5
Lysine	1.280 ± 0.067	1.200-1.370	5
Methionine	0.436 ± 0.165	0.306-0.699	5
Phenylalanine	0.938 ± 0.158	0.665-1.05	5
Threonine	0.855 ± 0.035	0.824-0.898	5
Tryptophan	0.277 ± 0.221	0.156-0.671	5
Tyrosine	0.618 ± 0.086	0.564-0.769	5
Valine	1.108 ± 0.043	1.050-1.170	5
Essential Fatty Acids (percent of total diet)			
Linoleic	2.290 ± 0.313	1.83-2.52	5
Linolenic	0.258 ± 0.040	0.210-0.308	5
Vitamins			
Vitamin A (IU/kg)	12,423 ± 4,794	3,600-24,000	26
Vitamin D (IU/kg)	4,450 ± 1,382	3,000-6,300	4
α-Tocopherol (ppm)	43.58 ± 6.92	31.1-48.0	5
Thiamine (ppm)	16.96 ± 3.40	12.0-27.0	26
Riboflavin (ppm)	7.6 ± 0.85	6.10-8.20	5
Niacin (ppm)	97.8 ± 31.68	65.0-150.0	5
Pantothenic acid (ppm)	30.06 ± 4.31	23.0-34.0	5
Pyridoxine (ppm)	7.68 ± 1.31	5.60-8.8	5
Folic acid (ppm)	2.62 ± 0.89	1.80-3.7	5
Biotin (ppm)	0.254 ± 0.053	0.19-0.32	5
Vitamin B_{12} (ppb)	24.21 ± 12.66	10.6-38.0	5
Choline (ppm)	3,122 ± 416.8	2,400-3,430	5
Minerals			
Calcium (percent)	1.28 ± 0.11	1.11-1.54	26
Phosphorus (percent)	0.97 ± 0.05	0.89-1.10	26
Potassium (percent)	0.900 ± 0.098	0.772-0.971	3
Chloride (percent)	0.513 ± 0.114	0.380-0.635	5
Sodium (percent)	0.323 ± 0.043	0.258-0.371	5
Magnesium (percent)	0.167 ± 0.012	0.151-0.181	5
Sulfur (percent)	0.304 ± 0.064	0.268-0.420	5
Iron (ppm)	410.3 ± 94.04	262.0-523.0	5
Manganese (ppm)	90.29 ± 7.15	81.7-99.4	5
Zinc (ppm)	52.78 ± 4.94	46.1-58.2	5
Copper (ppm)	10.72 ± 2.76	8.09-15.39	5
Iodine (ppm)	2.95 ± 1.05	1.52-3.82	4
Chromium (ppm)	1.85 ± 0.25	1.44-2.09	5
Cobalt (ppm)	0.681 ± 0.14	0.490-0.780	4

TABLE F4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminants	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.51 ± 0.15	0.17-0.77	26
Cadmium (ppm) (a)	<0.10		26
Lead (ppm)	0.76 ± 0.63	0.33-3.37	26
Mercury (ppm) (a)	<0.05		26
Selenium (ppm)	0.30 ± 0.07	0.13-0.42	26
Aflatoxins (ppb) (a)	<5.0		26
Nitrate nitrogen (ppm) (b)	8.66 ± 4.49	0.10-22.0	26
Nitrite nitrogen (ppm) (b)	2.05 ± 2.04	0.10-7.20	26
BHA (ppm) (c)	4.31 ± 4.70	2.00-17.0	26
BHT (ppm) (c)	2.59 ± 2.53	1.00-12.0	26
Aerobic plate count (CFU/g) (d)	40,765 ± 33,607	4,900-130,000	26
Coliform (MPN/g) (e)	46.12 ± 122.68	<3.00-460	26
E. coli (MPN/g)	<3.00		26
Total nitrosamines (ppb) (f)	5.16 ± 5.84	1.70-30.90	26
N-Nitrosodimethylamine (ppb) (f)	4.13 ± 5.83	0.80-30.00	26
N-Nitrosopyrrolidine (ppb) (f)	1.03 ± 0.25	0.81-1.00	26
Pesticides (ppm)			
α-BHC (a,g)	<0.01		26
β-BHC (a)	<0.02		26
γ-BHC (a)	<0.01		26
δ-BHC (a)	<0.01		26
Heptachlor (a)	<0.01		26
Aldrin (a)	<0.01		26
Heptachlor epoxide (a)	<0.01		26
DDE (a)	<0.01		26
DDD (a)	<0.01		26
DDT (a)	<0.01		26
HCB (a)	<0.01		26
Mirex (a)	<0.01		26
Methoxychlor (a)	<0.05		26
Dieldrin (a)	<0.01		26
Endrin (a)	<0.01		26
Telodrin (a)	<0.01		26
Chlordane (a)	<0.05		26
Toxaphene (a)	<0.1		26
Estimated PCBs (a)	<0.2		26
Ronnel (a)	<0.01		26
Ethion (a)	<0.02		26
Trithion (a)	<0.05		26
Diazinon (a)	<0.1		26
Methyl parathion (a)	<0.02		26
Ethyl parathion (a)	<0.02		26
Malathion (h)	0.10 ± 0.09	0.05-0.45	26
Endosulfan I (a)	<0.01		26
Endosulfan II (a)	<0.01		26
Endosulfan sulfate (a)	<0.03		26

(a) All values were less than the detection limit, given in the table as the mean.
(b) Source of contamination: alfalfa, grains, and fish meal
(c) Source of contamination: soy oil and fish meal
(d) CFU = colony-forming unit
(e) MPN = *most probable number*
(f) All values were corrected for percent recovery.
(g) BHC = hexachlorocyclohexane or benzene hexachloride
(h) Fourteen lots contained more than 0.05 ppm.

APPENDIX G

CHEMICAL CHARACTERIZATION AND GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS OF TETRANITROMETHANE FOR THE TOXICOLOGY STUDIES

APPENDIX G. CHEMICAL CHARACTERIZATION

PROCUREMENT AND CHARACTERIZATION OF TETRANITROMETHANE

Tetranitromethane was obtained in four lots; lot nos. TNM-80-154 and TNM-80-294 were from Hummel Chemical Co., Inc. (South Plainfield, NJ), and lot nos. F101882 and F081882 were from Fluorochem, Inc. (Azusa, CA) (Table G1). Purity and identity analyses of all lots of the bulk chemical were conducted at Midwest Research Institute (MRI) (Kansas City, MO) except for lot TNM-80-154, which was used only in the 14-day studies. MRI reports on the analyses performed in support of the tetranitromethane studies are on file at the National Institute of Environmental Health Sciences.

The study material was a clear, colorless, slightly viscous liquid. The identity of the lots analyzed was confirmed by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. Infrared spectra were consistent with those expected for the structure and with literature spectra of tetranitromethane (Sadtler Standard Spectra). Nuclear magnetic resonance and visible/ultraviolet spectra were consistent with those expected for the structure of tetranitromethane. (Representative spectra are presented in Figures G1 through G4.)

The purity of the analyzed lots was determined by titration, thin-layer chromatography, and gas chromatography. Titration was carried out by dissolving the study material in excess aqueous potassium iodide and titrating the liberated iodine with 0.1 N sodium thiosulfate using a starch indicator. Thin-layer chromatography was performed on silica gel 60 F-254 plates with solvent systems of either hexane:methylene chloride (90:10) or isooctane:ether (90:10). Visualization was with ultraviolet light (254 nm) and a sodium hydroxide spray observed under visible and ultraviolet light. Gas chromatographic analysis was performed with a thermal conductivity detector, a helium carrier at 70 ml/minute, and either a 10% SP2100 column (system 1) or a 5% SP1000 column (system 2).

For lot no. TNM-80-294, a purity of 100.3% was determined by titration. No impurities were detected by either thin-layer chromatographic system. No impurities having areas 0.1% or greater relative to the major peak area were detected by either gas chromatographic system.

TABLE G1. IDENTITY AND SOURCE OF TETRANITROMETHANE USED IN THE INHALATION STUDIES

Fourteen-Day Studies	Thirteen-Week Studies	One-Year Study	Two-Year Studies
Lot Numbers			
TNM-80-154	TNM-80-294	TNM-80-294	TNM-80-294; F101882; F081882
Date of Initial Use			
12/3/80	5/19/81	3/24/82	TNM-80-294--3/13/82 F101882--5/2/83 F081882--2/21/84
Supplier			
Hummel Chemical Co. Inc. (South Plainfield, NJ)	Same as 14-d studies	Same as 14-d studies	TNM-80-294--same as 14-d studies; F101882 and F081882--Fluorochem Inc. (Azusa, CA)

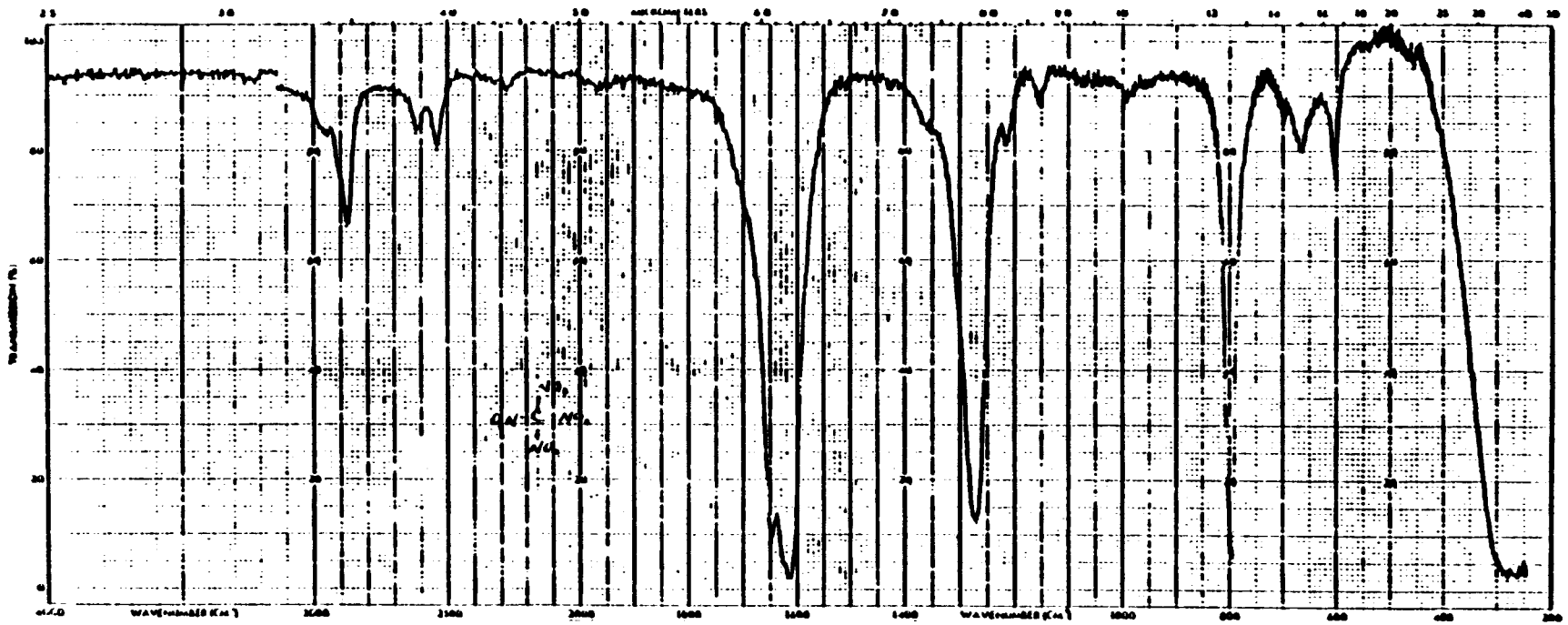

FIGURE G1. INFRARED ABSORPTION SPECTRUM OF TETRANITROMETHANE (LOT NO. TNM-80-294)

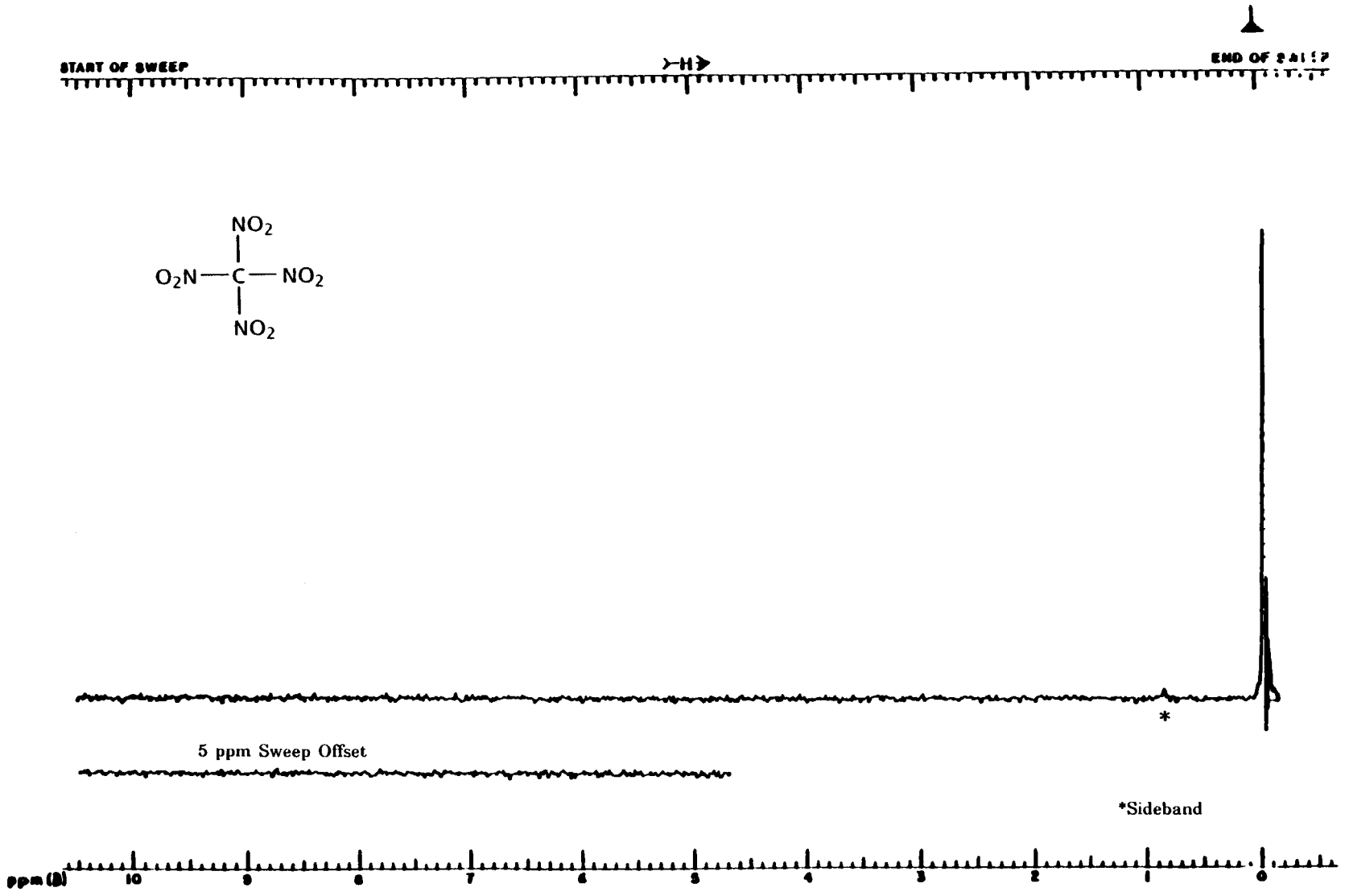

FIGURE G2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF TETRANITROMETHANE (LOT NO. TNM-80-294)

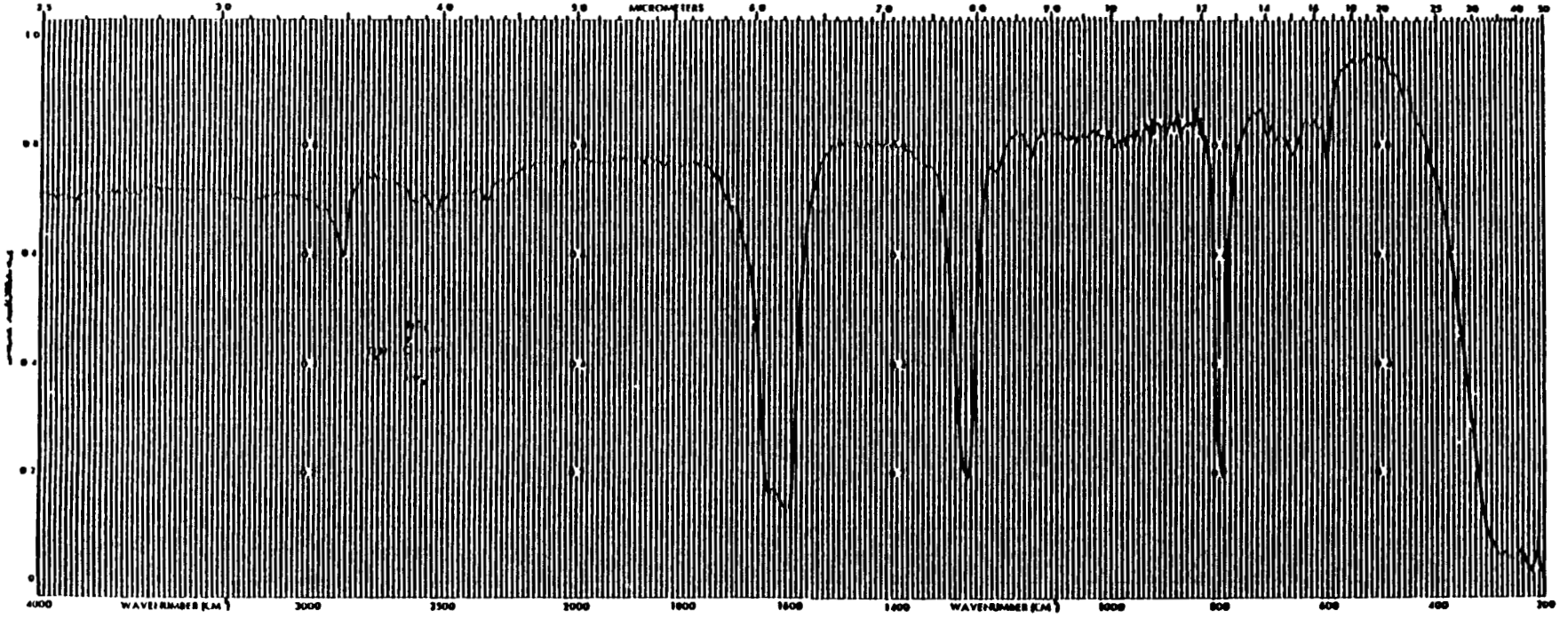

FIGURE G3. INFRARED ABSORPTION SPECTRUM OF TETRANITROMETHANE (LOT NO. F081882)

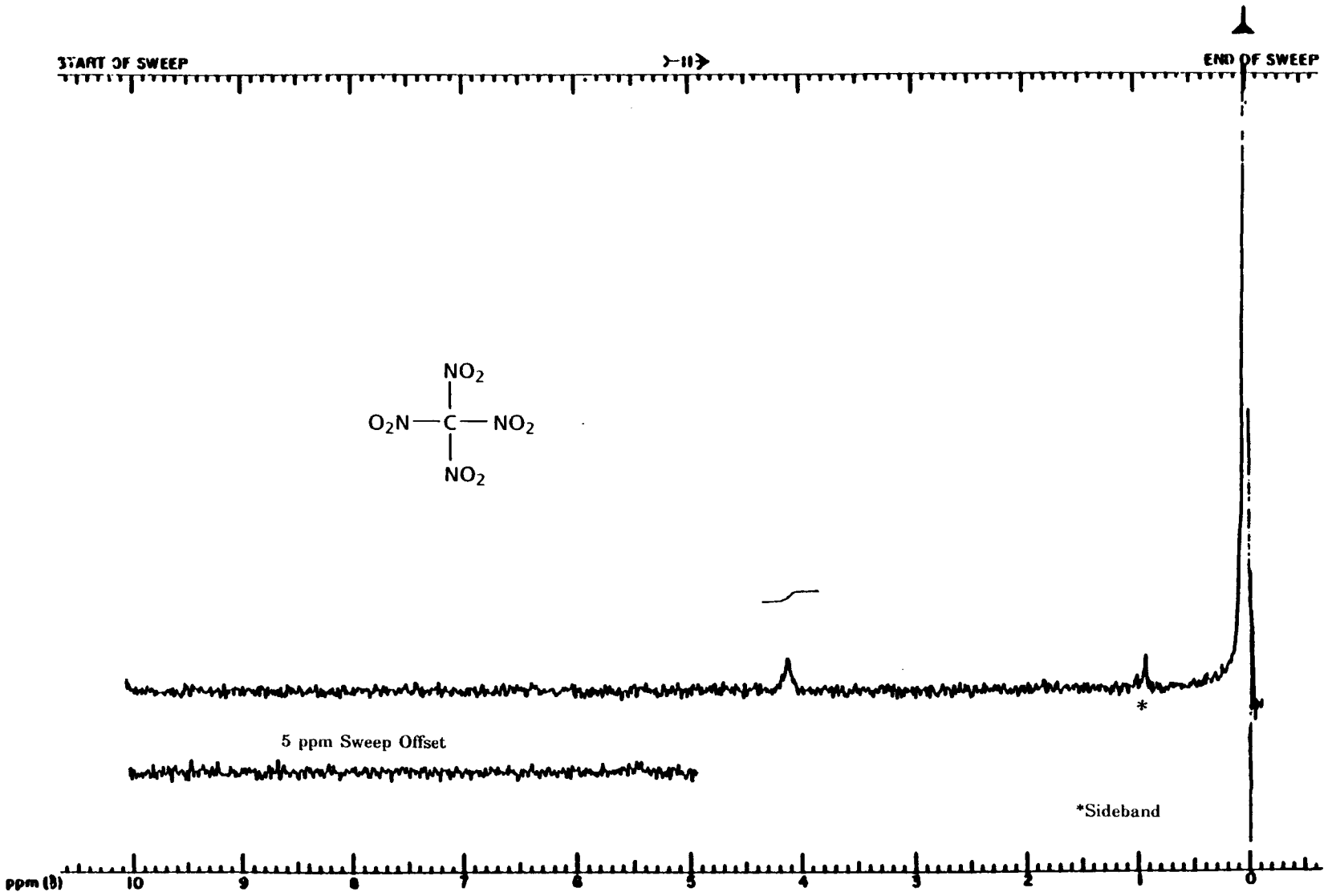

FIGURE G4. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF TETRANITROMETHANE (LOT NO. F081882)

For lot no. F101882, a purity of 100.4% was determined by titration. No impurities were detected by either thin-layer chromatographic system. Gas chromatographic system 1 indicated three impurities, with a combined area 0.45% relative to the major peak. Gas chromatographic system 2 indicated two impurities, with a combined relative area of 0.59%.

For lot no. F081882, a purity of 100.4% was indicated by titration. No impurities were detected by either thin-layer chromatographic system or by gas chromatographic system 2; one impurity, with a relative area of 0.1%, was detected by gas chromatographic system 1.

Stability studies performed by gas chromatography with the same column as previously described for system 1, with methylene chloride as an internal standard, indicated that tetranitromethane was stable as a bulk chemical when stored protected from light at temperatures up to 25° C. During these studies, the bulk chemical was stored at 5° C. Periodic analyses by gas chromatography and iodometric titration indicated no notable degradation of the study material throughout the studies.

GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS

Vapor Generation System

Tetranitromethane vapor was generated at room temperature from a gas dispersion bottle by bubbling nitrogen through the liquid. The vapor entered the airstream at the top of the chamber (Hazleton 2000®, Lab Products, Inc.) and was mixed and diluted with air in the chamber plenum before entering the chamber. During the 1- and 2-year studies, tetranitromethane vapor and the carrier nitrogen were transferred to secondary dilution flasks and further diluted with filtered nitrogen and channeled through stainless steel lines to the appropriate intake port of the study chamber where chamber intake air diluted the vapors to the desired concentration (Table G2). An individual generation system contained within an isolation box specially designed to operate under negative pressure was used for each exposure chamber.

TABLE G2. GENERATION OF CHAMBER CONCENTRATIONS IN THE INHALATION STUDIES OF TETRANITROMETHANE

Fourteen-Day Studies	Thirteen-Week Studies	One-Year Study	Two-Year Studies
Tetranitromethane was evaporated at room temperature from a gas dispersion bottle by nitrogen. Tetranitromethane vapor entered the airstream at the top of the chamber	Same as 14-d studies	Similar to 14-d studies except that the tetranitromethane vapor was further diluted with nitrogen in secondary flasks before final dilution with chamber intake air	Same as 1-y study

Vapor Concentration Monitoring

The concentration of tetranitromethane in the study chambers was monitored by a Wilks Miran 1A-CVF Infrared Process Analyzer (14-day studies) or a Miran® II Infrared Gas Analyzer (13-week and 2-year studies). The analytical and reference wavelengths were 7.04 and 4.90 μm, respectively. The gas analyzers were standardized once per day against air containing known tetranitromethane concentrations prepared by delivering accurately measured amounts of tetranitromethane into Tedlar gas sampling bags. Samples of study chamber atmosphere were drawn directly from the chambers and pulled into the gas analyzer. During the 1- and 2-year studies, samples of each study atmosphere and control atmosphere were analyzed every 10-15 minutes. The concentrations in the 0.5-ppm chamber were corrected for the slight effect of water vapor on the measured concentrations. The distribution of the mean daily concentrations in the chambers is summarized in Table G3.

TABLE G3. DISTRIBUTION OF MEAN DAILY CONCENTRATIONS OF TETRANITROMETHANE DURING THE TWO-YEAR INHALATION STUDIES

Range of Concentration (percent of target)	Number of Days Mean Within Specified Range		
	0.5 ppm	2 ppm	5 ppm
>120	0	0	0
110-120	21	(a) 3	0
		(b) 2	
90-110	464	(a) 491	495
		(b) 493	
80-90	10	(a) 1	0
Not exposed (c)	2	2	2

(a) Rats
(b) Mice
(c) Number of days animals not exposed because of equipment failure/analytical malfunctions

APPENDIX H

GENETIC TOXICOLOGY

OF TETRANITROMETHANE

APPENDIX H. GENETIC TOXICOLOGY

METHODS

Salmonella Protocol: Testing was performed as reported by Ames et al. (1975) with modifications listed below and described in greater detail by Zeiger et al. (1987) and Mortelmans et al. (1986). Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). The study chemical was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37° C before the addition of soft agar supplemented with L-histidine and D-biotin and subsequent plating on minimal glucose agar plates. Incubation was continued for an additional 48 hours.

Chemicals were tested in four strains. Each test consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of the study chemical. The high dose was limited by toxicity or solubility but did not exceed 215 µg/plate. All negative assays were repeated, and all positive assays were repeated under the conditions that elicited the positive response.

A positive response was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response was defined as an increase in revertants which was not dose related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A response was considered negative when no increase in revertant colonies was observed after chemical treatment.

Chinese Hamster Ovary Cytogenetics Assays: Testing was performed as reported by Galloway et al. (1985) and is described briefly below. Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). Chemicals were tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations both in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine (BrdU)-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of the study chemical; the high dose was limited by toxicity or solubility but did not exceed 5 mg/ml.

In the SCE test without S9, CHO cells were incubated for 26 hours with the study chemical in McCoy's 5A medium supplemented with 10% fetal bovine serum, L-glutamine (2 mM), and antibiotics. BrdU was added 2 hours after culture initiation. After 26 hours, the medium containing the study chemical was removed and replaced with fresh medium plus BrdU and colcemid, and incubation was continued for 2 more hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with the chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no study chemical; incubation proceeded for an additional 26 hours, with colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9.

In the chromosomal aberration test without S9, cells were incubated in McCoy's 5A medium with the study chemical for 8 hours; colcemid was added, and incubation was continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the chromosomal aberration test with S9, cells were treated with the study chemical and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

For the SCE test, if significant chemical-induced cell cycle delay was seen, incubation time was lengthened to ensure a sufficient number of scorable cells. The harvest time for the chromosomal

aberration test was based on the cell cycle information obtained in the SCE test; if cell cycle delay was anticipated, the incubation period was extended approximately 5 hours.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind, and those from a single test were read by the same person. For the SCE test, 50 second-division metaphase cells were usually scored for frequency of SCEs per cell from each dose; 200 first-division metaphase cells were scored at each dose for the chromosomal aberration test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. Chromosomal aberration data are presented as percentage of cells with aberrations. As with SCEs, both the dose-response curve and individual dose points were statistically analyzed. A statistically significant ($P<0.003$) trend test or a significantly increased dose point ($P<0.05$) was sufficient to indicate a chemical effect.

RESULTS

Tetranitromethane was tested for mutagenicity in four strains of *S. typhimurium* according to a preincubation protocol with concentrations of 0.03-215 µg/plate in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Zeiger et al., 1987; Table H1). Mutagenic activity was observed in strains TA98, TA100, and TA1535 with and without S9, but no increase in mutant colonies occurred in strain TA1537. In cytogenetic tests with CHO cells, tetranitromethane induced SCEs in the absence, but not the presence, of Aroclor 1254-induced male Sprague Dawley rat liver S9 (Table H2). In the second trial without S9, a delayed harvest protocol was used to offset chemical-induced cell cycle delay at the two highest doses, which had also produced a positive response; positive responses occurred at lower doses in the first trial without S9, where normal culture times were used. Chromosomal aberrations were also induced in CHO cells treated with tetranitromethane, but in contrast to the SCE results, positive responses occurred only in the presence of S9 (Table H3); standard harvest times were used for these cultures.

TABLE H1. MUTAGENICITY OF TETRANITROMETHANE IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/Plate (b)					
		−S9		+S9 (hamster)		+S9 (rat)	
		Trial 1	Trial 2	10%	30%	10%	30%
TA100	0	124 ± 11.9	122 ± 7.9	130 ± 6.1	123 ± 4.8	142 ± 1.9	130 ± 3.8
	0.03	127 ± 11.9					
	0.1	147 ± 4.0					
	0.3	138 ± 2.7	139 ± 3.8				
	1	164 ± 7.2	163 ± 2.7				
	2		203 ± 6.0				
	2.5		223 ± 10.7				
	3.3	(c) 299 ± 3.1	(c) 255 ± 7.9		113 ± 9.0		137 ± 8.2
	10			150 ± 1.8	132 ± 4.4	158 ± 9.5	138 ± 8.8
	20			213 ± 12.8			
	33			373 ± 21.2	300 ± 13.9	190 ± 4.8	203 ± 9.5
	50			(c) 604 ± 7.1		227 ± 4.3	
	75			(c) 257 ± 12.5		387 ± 15.8	
	100				(c) 419 ± 65.0	418 ± 17.8	(c) 357 ± 17.8
	150					507 ± 11.3	
	215				Toxic		(c) 661 ± 27.0
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control (d)		2,202 ± 24.8	1,371 ± 29.9	3,128 ± 44.7	2,331 ± 12.0	1,547 ± 31.0	1,150 ± 88.1
TA1535	0	28 ± 3.6	18 ± 1.2	7 ± 1.5	12 ± 2.5	13 ± 2.5	11 ± 0.7
	0.03	21 ± 0.3					
	0.1	27 ± 1.5					
	0.3	28 ± 1.7	25 ± 0.9				
	1	(c) 33 ± 1.5	28 ± 5.2				
	2		48 ± 4.0				
	2.5		62 ± 2.1				
	3.3	(c) 73 ± 6.8	67 ± 5.0		14 ± 1.2		15 ± 0.9
	10			12 ± 1.2	23 ± 3.2	16 ± 1.8	22 ± 2.3
	20			57 ± 7.4			
	33			184 ± 2.9	165 ± 9.8	42 ± 18.2	50 ± 6.6
	50			299 ± 8.4		87 ± 8.1	
	75			(c) 123 ± 3.9		182 ± 11.6	
	100				Toxic	202 ± 21.9	(c) 191 ± 9.0
	150					276 ± 9.5	
	215				Toxic		(c) 200 ± 9.0
Trial summary		Equivocal	Positive	Positive	Positive	Positive	Positive
Positive control (d)		1,452 ± 99.3	1,012 ± 33.3	167 ± 2.6	148 ± 9.0	85 ± 4.3	89 ± 21.1

TABLE H1. MUTAGENICITY OF TETRANITROMETHANE IN *SALMONELLA TYPHIMURIUM* (Continued)

Strain	Dose (µg/plate)	Revertants/Plate (b)		
		−S9	+30% S9 (hamster)	+30% S9 (rat)
TA1537	0	17 ± 2.2	21 ± 4.0	15 ± 1.2
	0.03	17 ± 0.3		
	0.1	19 ± 1.2		
	0.3	18 ± 2.7		
	1	21 ± 2.6		
	3.3	(c) 8 ± 0.9	23 ± 2.0	16 ± 1.3
	10		18 ± 3.2	18 ± 0.7
	33		17 ± 4.0	20 ± 0.9
	100		Toxic	19 ± 2.9
	215		Toxic	(c) 11 ± 3.8
Trial summary		Negative	Negative	Negative
Positive control (d)		278 ± 11.5	164 ± 6.4	144 ± 3.6

		−S9		+S9 (hamster)		+S9 (rat)	
		Trial 1	Trial 2	10%	30%	10%	30%
TA98	0	17 ± 0.7	19 ± 0.9	28 ± 2.3	25 ± 1.2	34 ± 1.5	36 ± 1.9
	0.03	23 ± 1.9					
	0.1	19 ± 2.9					
	0.3	21 ± 0.6	18 ± 2.8				
	1	26 ± 0.7	26 ± 2.9				
	2		32 ± 6.9				
	2.5		42 ± 11.8				
	3.3	(c) 78 ± 9.3	(c) 24 ± 4.9		33 ± 3.8		29 ± 1.3
	10			29 ± 2.9	30 ± 2.9	30 ± 1.0	31 ± 4.7
	20			35 ± 5.2			
	33			28 ± 5.6	48 ± 4.1	36 ± 1.9	35 ± 3.3
	50			51 ± 8.4		46 ± 1.5	
	75			86 ± 8.2		45 ± 3.5	
	100				Toxic	50 ± 3.5	60 ± 4.7
	150					(c) 70 ± 5.4	
	215				Toxic		Toxic
Trial summary		Positive	Weakly positive	Positive	Equivocal	Weakly positive	Equivocal
Positive control (d)		1,826 ± 84.5	1,850 ± 22.4	2,581 ± 40.1	1,975 ± 22.1	1,228 ± 9.8	1,115 ± 45.6

(a) Study performed at EG&G Mason Research Institute. The detailed protocol and data are presented in Zeiger et al. (1987). Cells and study compound or solvent (dimethyl sulfoxide) were incubated in the absence of exogenous metabolic activation (−S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 µg/plate dose is the solvent control.
(b) Revertants are presented as mean ± standard error from three plates.
(c) Slight toxicity
(d) Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA1537.

TABLE H2. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY TETRANITROMETHANE (a)

Compound	Dose (µg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hours in BrdU	Relative SCEs/ Chromosome (percent) (b)
−S9 (c)								
Trial 1--Summary: Positive								
Dimethyl sulfoxide		50	1,030	497	0.48	9.9	25.5	
Tetranitromethane	0.50	50	1,016	530	0.52	10.6	25.5	8.11
	1.7	50	1,028	627	0.60	12.5	25.5	*26.40
	5	50	1,035	813	0.78	16.3	25.5	*62.79
Mitomycin C	0.001	50	1,029	620	0.60	12.4	25.5	24.87
	0.01	5	105	245	2.33	49.0	25.5	383.57
Trend test: P<0.001								
Trial 2--Summary: Positive								
Dimethyl sulfoxide		25	513	203	0.39	8.1	26.0	
Tetranitromethane	2.5	25	516	223	0.43	8.9	26.0	9.21
	5	25	512	332	0.64	13.3	(d) 33.1	*63.86
	7.5	25	511	396	0.77	15.8	(d) 33.1	*95.84
Mitomycin C	0.001	25	518	256	0.49	10.2	26.0	24.89
	0.01	5	102	200	1.96	40.0	26.0	395.51
Trend test: P<0.001								
+S9 (e) Summary: Negative								
Dimethyl sulfoxide		50	1,024	494	0.48	9.9	25.5	
Tetranitromethane	1.7	50	1,032	467	0.45	9.3	25.5	−6.20
	5	50	1,011	499	0.49	10.0	25.5	2.31
	16.8	50	1,035	484	0.46	9.7	25.5	−3.07
Cyclophosphamide	0.4	50	1,018	655	0.64	13.1	25.5	33.37
	2	5	103	195	1.89	39.0	25.5	292.44
Trend test: P=0.524								

(a) Study performed at Litton Bionetics, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent as described in (c) and (e) below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air dried, and stained.
(b) Percentage change in the value of SCEs/chromosome for exposed culture compared with that for solvent control culture. An increase of 20% or more was considered to be a significant response.
(c) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent for 2 hours at 37° C. Then BrdU was added, and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 hours.
(d) Because some chemicals induce a delay in the cell division cycle, harvest times are occasionally extended to maximize the proportion of second division cells available for analysis.
(e) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with colcemid present for the final 2-3 hours. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.
*P<0.05

TABLE H3. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY TETRANITROMETHANE (a)

Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
−S9 (b)				
Harvest time: 20 hours (d)				
Dimethyl sulfoxide				
	200	3	0.02	1.0
Tetranitromethane				
1.1	200	0	0.00	0.0
1.5	200	2	0.01	1.0
3.7	135	2	0.01	1.5
Summary: Negative				
Mitomycin C				
0.05	200	90	0.45	19.5
0.08	25	29	1.16	68.0
Trend test: P = 0.251				
+S9 (c)				
Trial 1--Harvest time: 12 hours (d)				
Dimethyl sulfoxide				
	200	3	0.02	1.5
Tetranitromethane				
8	200	4	0.02	1.5
19.9	200	2	0.01	1.0
39.7	200	34	0.17	*12.0
Summary: Weakly positive				
Cyclophosphamide				
7.5	200	20	0.10	8.5
37.5	25	25	1.00	40.0
Trend test: P < 0.001				
Trial 2--Harvest time: 12 hours (d)				
Dimethyl sulfoxide				
	100	2	0.02	2.0
Tetranitromethane				
10	100	4	0.04	4.0
20	100	13	0.13	*11.0
Summary: Weakly positive				
Cyclophosphamide				
7.5	100	9	0.09	8.0
37.5	25	23	0.92	44.0
Trend test: P = 0.003				

(a) Study performed at Litton Bionetics, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent as indicated in (b) and (c). Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.
(b) In the absence of S9, cells were incubated with study compound or solvent for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 hours followed by harvest.
(c) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation before harvest. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.
(d) Because of significant chemical-induced cell cycle delay, incubation time prior to addition of colcemid was lengthened to provide sufficient metaphases at harvest.
*P < 0.05

APPENDIX I

ACTIVATION OF THE K-*ras* PROTOONCOGENE IN LUNG TUMORS FROM RATS AND MICE CHRONICALLY EXPOSED TO TETRANITROMETHANE

Stowers, S.J.; Glover, P.L.; Reynolds, S.H.; Boone, L.R.; Maronpot, R.R.; Anderson, M.W. (1987) Cancer Res. 47:3212-3219

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Activation of the K-*ras* Protooncogene in Lung Tumors from Rats and Mice Chronically Exposed to Tetranitromethane

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ABSTRACT

Dominant transforming genes were detected in lung tumors from Fischer 344 rats and C57BL/6 × C3H F_1 mice chronically exposed by inhalation to tetranitromethane, a highly volatile compound used in several industrial processes. The rat lung neoplasms were classified as adenocarcinomas, squamous cell carcinomas (epidermoid carcinomas), or adenosquamous carcinomas. The mouse lung tumors were classified as papillary adenocarcinomas or adenomas. In both species, the tumors were morphologically similar to lung tumors in humans. The transfection assay using NIH/3T3 mouse fibroblasts detected transforming genes in 74% (14 of 19) of the rat lung tumors and in 100% (4 of 4) of the mouse lung tumors. Southern blot analysis indicated that transforming gene was an activated K-*ras* protooncogene in both species. The first exon of the K-*ras* gene in normal DNA and in DNA from two cell lines transformed by tumor DNA was compared by cloning and sequencing the gene. Experiments showed that there was a GC→AT transition in the second base of the 12th codon of the K-*ras* oncogene in the two transfectant DNAs. Oligonucleotide hybridization indicated that all of the rat and mouse transfectants had this activating lesion. Additional tumor DNA was then tested for the presence of a mutated allele with the GC→AT transition. All of the rat tumors tested and all of the mouse tumors tested had this mutation present. Hybridization using the normal oligonucleotide sequence around the 12th codon indicated that the normal allele was also present in the majority of the tumors, suggesting that the loss of normal allele is not necessary for the development of neoplasia. One rat lung tumor had no normal allele present, possibly suggesting that this tumor could have been in a more advanced stage than the other tumors. This is the first study to detect activated protooncogenes in rodent tumors induced under conditions which mimic human exposure to a chemical in the workplace. Tetranitromethane may exert its carcinogenic action by both activation of the K-*ras* oncogene and stimulation of cell proliferation by its irritant properties.

INTRODUCTION

Recent studies suggest that the activation of protooncogenes by genetic alterations may play a role in leading a cell to neoplastic development. These genetic alterations include gross chromosomal rearrangements, amplification of genes, and point mutations. Oncogenes that have been shown to acquire transforming activity by point mutation in their coding sequence include members of the *ras* oncogene family, the H-*ras*, K-*ras*, and the N-*ras* (1-13) and the *neu* oncogene (14). The activation of the *ras* family of genes usually occurs via a point mutation at the 12th, 13th, or 61st codons in human tumors and tumor cell lines (1-13). Studies in a variety of animal model systems have shown that specific activation of a protooncogene by point mutation can be caused by chemical or physical insult (1-8).

Animal model systems for carcinogenesis have provided a good means to study protooncogene activation in tumor development. The H-*ras* protooncogene has reproducibly been found activated in rat mammary carcinomas induced by a single injection of *N*-methyl-*N*-nitrosourea given during sexual development (3). The H-*ras* protooncogene has also been found activated in mouse skin papillomas and carcinomas induced by DMBA[2] followed by phorbol ester (12-*O*-tetradecanoylphorbol-13-acetate) promotion (1, 2, 7). In both models, the H-*ras* protooncogene was found to be activated in 90-100% of all of the tumors examined. Other studies have found K-*ras* and N-*ras* activation in X-ray- or *N*-methyl-*N*-nitrosourea-induced mouse thymomas and in rat mesenchymal kidney tumors induced by treatment with methyl(methoxy-methyl)nitrosamine (4, 5, 15). One conclusion from these studies is that exposure to carcinogens either by relatively high single or multiple doses causes changes in the DNA resulting in activation of oncogenes. However, no studies have examined protooncogene activation in tumors that develop after long term, chronic exposure to chemicals. The identification of chemicals as potential human carcinogens is often made on the basis of long term rodent bioassays which are designed to consider route of human exposure and concentrations similar to those present in the environment, workplace or home.

In a recent bioassay conducted by the National Toxicology Program, chronic exposure to the industrial chemical TNM induced a high incidence of primary lung tumors in Fischer 344 rats and C57BL/6 × C3H F_1 (hereafter called B6C3F_1) mice.[3] TNM is a highly volatile compound used as a reagent in industrial nitrosating processes, as an oxidant in rocket fuel, and as an explosive when mixed with toluene (tetranitrotoluene). Because of its irritant properties, TNM has also been proposed as a war gas. The threshold limit for occupational exposure to TNM based on its irritant properties has been set at 1 ppm. In the bioassay, groups of 50 male and 50 female Fischer 344 rats or B6C3F_1 mice were exposed to TNM by inhalation for 6 h a day, 5 days a week for 2 years. The rats were exposed to 0, 2, and 5 ppm of TNM while the mice were exposed to 0, 0.5, and 1 ppm. Based on histomorphological examination, the TNM-induced primary lung tumors were adenomas, adenocarcinomas, squamous cell carcinomas, and adenosquamous carcinomas in rats and papillary adenomas and adenocarcinomas in mice. These tumors were morphologically similar to primary lung tumors in humans. The purpose of this study was to identify and characterize any activated oncogenes that might be present in lung tumors from rats and mice after chronic exposure to TNM.

MATERIALS AND METHODS

Lung Tumor Generation. Two-year toxicity and carcinogenicity studies of TNM were performed under National Toxicology Program

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[1] To whom requests for reprints should be addressed.

[2] The abbreviations used are: DMBA, 7,12-dimethylbenzanthracene; TNM, tetranitromethane.

[3] The National Toxicology Program has not yet completed its evaluation of the data collected during the studies with TNM. Therefore, the apparent association of TNM exposure with lung tumors in rats or mice should be considered preliminary, pending approval of the National Toxicology Program Technical Report on TNM by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee.

Contract NO1-ES-38042 from March 1982 to March 1984 at Midwest Research Institute, Kansas City, MO. Complete experimental details are contained in the Midwest Research Institute Report on Project 7801-E(1). TNM (99% pure) was generated using carrier grade nitrogen and a two-stage dilution system. Test atmospheres were monitored using a Miran II IR gas analyzer every 10–15 min during exposures. Groups of 50 male and 50 female Fischer 344 rats were exposed to 0, 2, or 5 ppm of TNM by inhalation for 6 h/day, 5 days/week for 2 years. Similar groups of $B6C3F_1$ mice were exposed to 0, 0.5, and 1 ppm. All animals received a complete necropsy, and tissues were collected for microscopic evaluation which was performed by Pathology Associates Ijamsville, MD. At necropsy lungs were inflated to normal inspiratory volume with 10% neutral buffered formalin and immersed in the same fixative. Hematoxylin- and eosin-stained paraffin sections were prepared according to routine pathological procedures. During the terminal sacrifice, lung tumors and normal lung tissue were collected for this study. At this time representative portions of selected rat and mouse lung tumors were fixed in 3% glutaraldehyde and subsequently processed for transmission electron microscopic examination.

DNA Isolation. High molecular weight DNA was isolated from normal or tumor tissues by using Pronase-sodium dodecyl sulfate lysis. Following phenol-chloroform extraction and ethanol precipitation, the DNA samples were treated with RNase and additional phenol-chloroform extractions and ethanol precipitation (16). The size of the DNA was checked on a 0.7% agarose gel.

Transfection Assay. High molecular weight DNA from the rat or mouse lung tumors was transfected onto NIH/3T3 mouse fibroblasts (30 μg/plate, four plates/sample) by the calcium phosphate precipitation method described previously (16). The cells were maintained with Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY) supplemented with 5% calf serum (Colorado Serum Co., Denver, CO) for 21 days until the foci were scored. Isolated foci were grown in 10% calf serum Dulbecco's modified Eagle's medium and stored as cell pellets until needed for DNA isolation and subsequent transfection or hybridizations.

Southern Blot Analysis. High molecular weight DNA was isolated, digested with *Hind*III (Boehringer-Mannheim, Indianapolis, IN), and electrophoresed on a 0.7% agarose gel (20 μg/lane). The DNA was then transferred to nitrocellulose (16). After baking and prehybridization, the blot was hybridized under stringent conditions (50% formamide/0.75 M NaCl/0.075 M sodium citrate; 42°C) to the *Sst*II-*Xba*I fragment containing the first, second, and part of the third exons (HiHi380) of the v-*kis* oncogene for rat DNA and the *Sst*II-*Hin*cII fragment (Oncor, Gaithersburg, MD) for the mouse DNA (17). The blot was washed to a final stringency of 0.2× sodium citrate solution-0.1% sodium dodecyl sulfate at 50°C. The blot was exposed to film overnight at −70°C with intensifying screens.

Cloning and Sequencing. Total normal rat DNA or transfectant DNA derived from an adenocarcinoma or a squamous cell carcinoma was digested with *Hind*III, ligated to phage λ Charon 28 *Hind*III arms, and packaged using the Promega Packagene System (Madison, WI). Positive plaques containing the first exon of the rat K-*ras* oncogene were identified by hybridization to the *Sst*II-*Sau*3A1 fragment (containing the first exon) of the v-*kis* oncogene (17). Phage from the positive plaques were grown and the size was checked by digestion with *Hind*III and electrophoresis on an agarose gel. Southern transfer and subsequent hybridization to the first exon probe confirmed the presence of the K-*ras* first exon 2.6-kilobase insert. This insert was subcloned into *Hind*III-cut pBR322. A restriction map was obtained by a combination of single and double digests of various enzymes. The first exon was localized to a 0.6-kilobase *Eco*RI-*Hind*III fragment. This fragment was then subcloned into M13mp19 for dideoxy sequencing using the BRL Cloning and Sequencing Kit (Bethesda, MD) (18).

Oligonucleotide Hybridization. *Hind*III- or *Eco*RI-digested DNA was electrophoresed on a 0.7% agarose gel, hybridized, and washed according to the method of Bos *et al.* (19) with the following modifications: 50% formamide was used in the hybridization buffer; gels were hybridized at 42°C; and the gels were washed to a final stringency of two 15-min washes at 62°C in 2× sodium citrate solution. After being wrapped in plastic wrap, the gels were exposed to film for 1–3 days. The sequences of the oligonucleotide probes used in these experiments are the normal sequence 5′-TTGGAGCTGGTGGCGTAGG-3′ from E. P. Reddy or the mutated sequence 5′-TTGGAGCTGATGGCGTAGG-3′ (OCS Laboratories, Denton, TX).

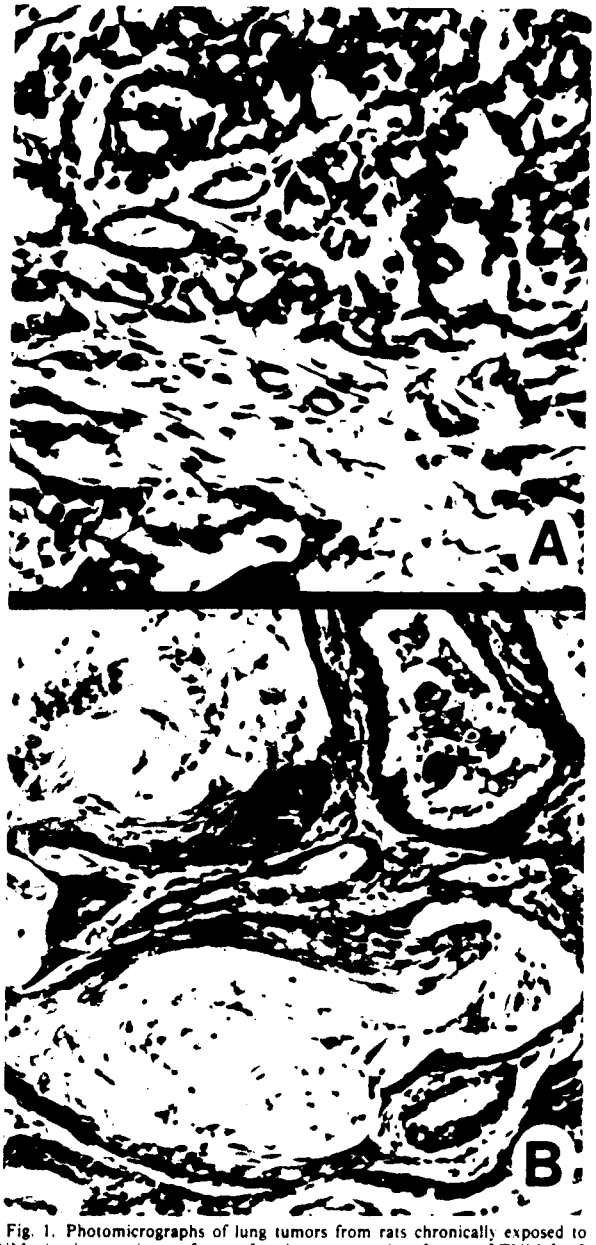

Fig. 1. Photomicrographs of lung tumors from rats chronically exposed to TNM. *A*, adenocarcinoma from a female rat exposed to 5 ppm of TNM for 2 years. Gland formation is evidence along with connective tissue proliferation. H & E. × 180. *B*, squamous cell carcinoma from a female rat exposed to 5 ppm of TNM for 2 years. There are irregular glands filled with necrotic cellular debris in the lower part of the photomicrograph. Squamous cells and keratin are present at the top of the figure. H & E. × 150.

RESULTS

Activated Oncogenes in Rat Lung Tumors

Tumor Generation. In contrast to the absence of primary lung tumors in controls, male and female rats exposed to TNM had a high incidence of benign and/or malignant lung tumors. There

Table 1 *Transforming genes in TNM-induced lung tumors in rats*

DNA source	Samples tested	Transforming genes (% positive)	Transformation efficiency (foci/μg DNA)
Primary adenocarcinoma	12	9/12 (75)	0.003–0.009
Squamous cell carcinoma	4	3/4 (75)	0.003
Adenosquamous carcinoma	3	2/3 (67)	0.006
Normal tissue	8	0/8 (0)	

DNA was isolated from rat lung tumors and transfected onto NIH/3T3 mouse fibroblasts by the calcium phosphate precipitation method described previously (6). The cells were maintained with Dulbecco's modified Eagle's medium (GIBCO) supplemented with 5% calf serum (Colorado Serum Co.) for 21 days until the foci were scored. Isolated foci were grown in 10% calf serum-Dulbecco's modified Eagle's medium for DNA isolation and subsequent transfection.

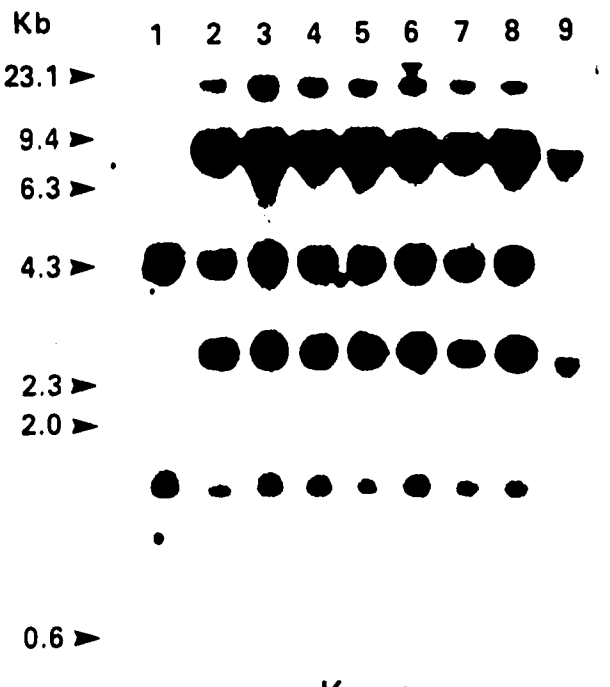

Fig. 2. High molecular weight DNA was isolated, digested with *Hind*III (Boehringer-Mannheim), and electrophoresed on a 0.7% agarose gel. Molecular weight standards from *Hind*III-digested wild-type λ DNA are noted at left. Bands at 7.4 and 2.6 kilobases (Kb) contain rat sequences that are homologous to the *Sst*II-*Xba*I fragment (HiHi380) of the v-*kis* oncogene (17). *Lane 1*, 20 μg of NIH 3T3 DNA; *Lanes 2–5*, 20 μg of secondary transfectant DNA generated initially from adenocarcinomas; *Lanes 6–7*, 20 μg of secondary transfectant DNA generated initially from squamous cell carcinomas; *Lane 8*, 20 μg of secondary transfectant DNA generated initially from an adenosquamous carcinoma; *Lane 9*, 20 μg normal rat DNA from the spleen.

was a dose-related increased incidence, increased multiplicity, and increased frequency of local invasion as well as distant metastases of the lung tumors in TNM-exposed rats. The earliest occurrence of lung tumors was observed in rats that died after 12 months of exposure to TNM. The benign lung tumors were solid bronchioalveolar adenomas (20, 21). The malignant tumors were adenocarcinomas usually with a significant amount of stromal proliferation (Fig. 1*A*), squamous cell carcinomas with abundant keratin formation (Fig. 1*B*), and adenosquamous carcinomas. Based on electron microscopic examination of some of the adenocarcinomas, the cells in some tumors were compatible with Clara cells and others with type II cells. Because of the small tumor size, no benign tumors were available for oncogene analysis from this bioassay.

Transfection Assay. Fourteen of 19 tumor DNA (74%) induced morphological transformation of the NIH/3T3 cells, indicating the presence of a dominant transforming gene (see Table 1). Individual tumor types had similar results: 75% of the primary adenocarcinoma DNA were positive; 75% of the squamous cell carcinoma DNA were positive; and 67% of the adenosquamous carcinoma DNA were positive. Eight samples of rat lung DNA obtained from the air-exposed controls were negative in this assay. The transforming frequency ranged from 0.012–0.036 foci/μg DNA for the first cycle of the transfection and was 10-fold higher for the second cycle. No histomorphological differences could be detected between those rat samples with or without transforming activity as detected by this assay.

Southern Analysis. Frequently, the transforming gene detected by the NIH/3T3 assay has been a mutated version of a member of the *ras* gene family (1–13, 19, 22–24). *Hind*III-digested rat transfectant DNA was tested for the presence of novel or amplified restriction fragments that hybridized to H-*ras*-, K-*ras*-, or N-*ras*-specific oncogene probes using Southern blot analysis. An activated H-*ras* or N-*ras* could not be detected in any of the transfectant DNA from the rat lung tumors induced by TNM (data not shown). As shown in Fig. 2, the K-*ras* probe hybridized to two *Hind*III fragments (at 7.4 and 2.6 kilobases) in each secondary transfectant DNA (Fig. 2, *Lanes 2–8*) in addition to the three NIH/3T3 mouse K-*ras* *Hind*III fragments (17.3, 3.6, and 1.6 kilobases in *Lanes 1–8*). These two novel bands appear to be amplified and comigrate with normal rat K-*ras* bands (*Lane 9*) suggesting that the transforming properties of the TNM lung tumor DNA were due to the transfer of an activated cellular homologue of the rat K-*ras* protooncogene into the NIH/3T3 cells.

Cloning and Sequencing. The most prominent lesion in activated K-*ras* protooncogenes to date has involved mutations in the 12th codon (4, 6, 9–11). Upon examination of TNM-activated K-*ras* protein products, it was found that these proteins comigrate with the normal *ras* proteins on a sodium dodecyl sulfate-polyacrylamide gel, which indicated no apparent mutation at the 12th or 61st codons (data not shown). To determine if there was a mutation present at the 12th codon of the K-*ras* oncogene in these rat lung tumors, the first exon of the normal rat K-*ras* protooncogene and the protooncogene activated in two TNM transfectant DNAs were cloned and the nucleotide sequences were determined. Total normal rat DNA or transfectant DNA derived from an adenocarcinoma and a squamous cell carcinoma was digested with *Hind*III and cloned into λ Charon 28 vector. Restriction mapping of the 2.6-kilobase *Hind*III fragment after subcloning into the plasmid pBR322 localized the first exon of the K-*ras* gene in normal rat DNA and the two transfectant DNAs to a 0.6-kb *Eco*RI-*Hind*III fragment. This fragment was then subcloned into M13mp19 for dideoxy sequencing (18). Only a single base difference between the normal rat and both TNM transfectant cloned sequences was found involving a GC→AT transition in the second base of the triplet coding for amino acid 12, changing glycine to aspartic acid. This GC→AT transition was seen in both of the cloned transfectants indicating that the activating lesions were the same regardless of the morphological appearance of the original tumors.

Oligonucleotide Hybridization. Normal and mutated radioactive oligonucleotide probes centered on the second base of the 12th codon were hybridized to the *Hind*III-digested transfectant DNA and cloned versions of the normal and mutated K-*ras* first exons to determine if more of the transfectants had this same activating lesions, GC→AT (see Fig. 3). There was no hybridization of the mutated oligonucleotide probe to DNA from the normal clone (Fig. 3*B*, *Lanes 10* and *12*) under

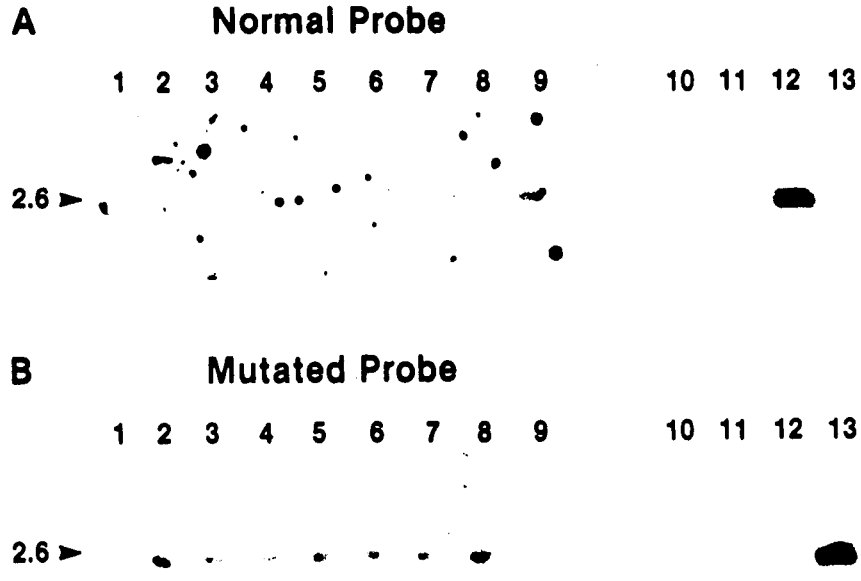

Fig. 3. Detection of the point mutation with a specific oligonucleotide. The oligonucleotides used in these experiments are the normal sequences 5'TTGGAGCTGGTGGCGTAGG-3' from E. P. Reddy or the mutated sequence 5'-TTGGAGCTGATGGCGTAGG-3' (OCS Laboratories). *A*, hybridization of the normal oligonucleotide probe to *Hind*III-digested DNA. *B*, hybridization of mutated oligonucleotide probe to *Hind*III-digested DNA. In *A* and *B*: *Lane 1*, 20 μg of NIH/3T3 DNA; *Lanes 2-8*, 20 μg of secondary transfectant DNA generated initially from rat adenocarcinomas or squamous cell carcinomas; *Lane 9*, 20 μg of normal rat DNA, *Lanes 10* and *12*, 0.3 and 3.0 ng of normal first exon clone in pBR322, respectively. *Lanes 11* and *13*, 0.3 and 3.0 ng of the mutated first exon clone in pBR322, respectively.

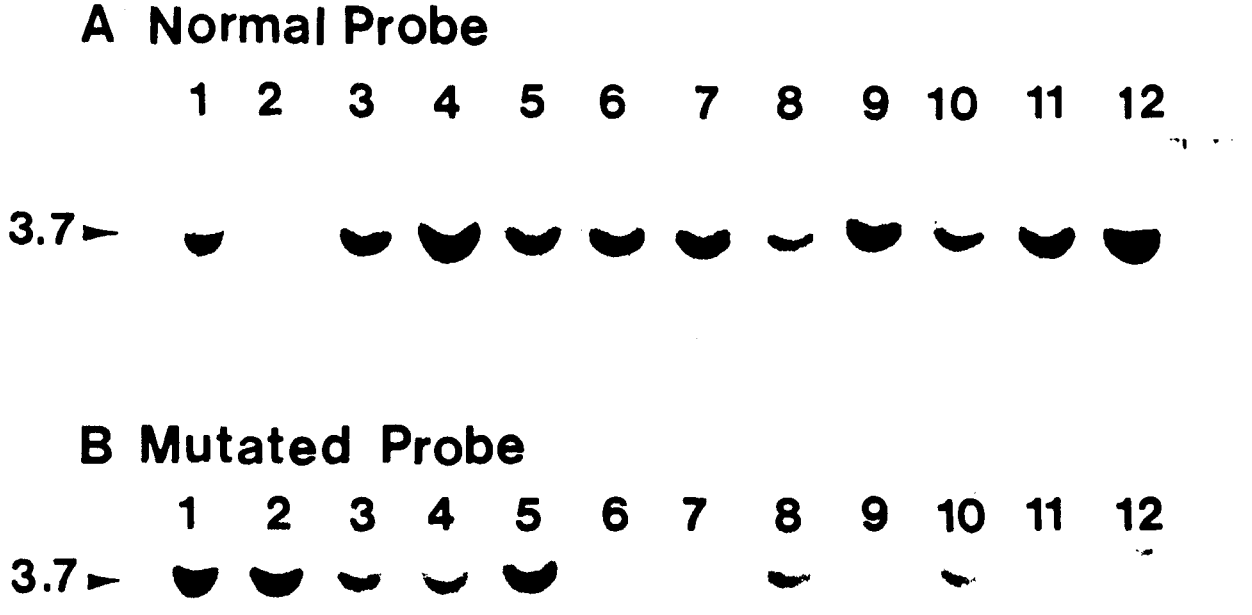

Fig. 4. Detection of mutated sequences in the K-*ras* oncogene in the original rat tumors. Rat tumors wee digested with EcoRI, run on a 0.7% agarose gel, and dried as described previously (19). *A*, hybridization of the normal probe to the rat tumor DNA. *B*, hybridization of the mutated probe to the rat tumor DNA. In *A* and *B*: *Lanes 1-5*, *10*, and *11*, 20 μg DNA from TNM-induced rat lung tumors that were positive on transfection: *Lanes 6-9*, 20 μg DNA from TNM-induced rat lung tumors that were negative on transfection; *Lane 12*, 20 μg DNA from normal rat lung.

conditions where strong hybridization was observed with the normal oligonucleotide probe (Fig. 3*A*, *Lanes 11* and *13*). In contrast, strong hybridization to the mutated transfectant clone was observed with the mutated (Fig. 3*B*, *Lanes 11* and *13*) but not the normal oligonucleotide probe (Fig. 3*A*, *Lanes 11* and *13*). The mutated oligonucleotide probe also bound to each of the seven TNM rat transfectant first exons (Fig. 3*B*, *Lanes 2-8*) and not to normal rat DNA (Fig. 3*B*, *Lane 9*) or to NIH/3T3 DNA (Fig. 3*B*, *Lane 1*). The normal oligonucleotide probe bound only to the normal K-*ras* first exon in the rat genomic DNA (Fig. 3*A*, *Lane 9*) and not to the transfectant DNA (Fig. 3*A*, *Lanes 2-8*). Taken together these data indicate that the same activating lesion is present in each of the transfectants derived from tumors induced by chronic exposure to TNM.

To see if the mutation could be detected in the tumor directly, seven tumor DNA that were positive on the transfection assay and four tumor DNA that were negative on the assay were examined by oligonucleotide hybridization. Complete digestion of rat lung tumor DNA was not possible with the restriction enzyme *Hind*III used to digest the transfectant DNA probably

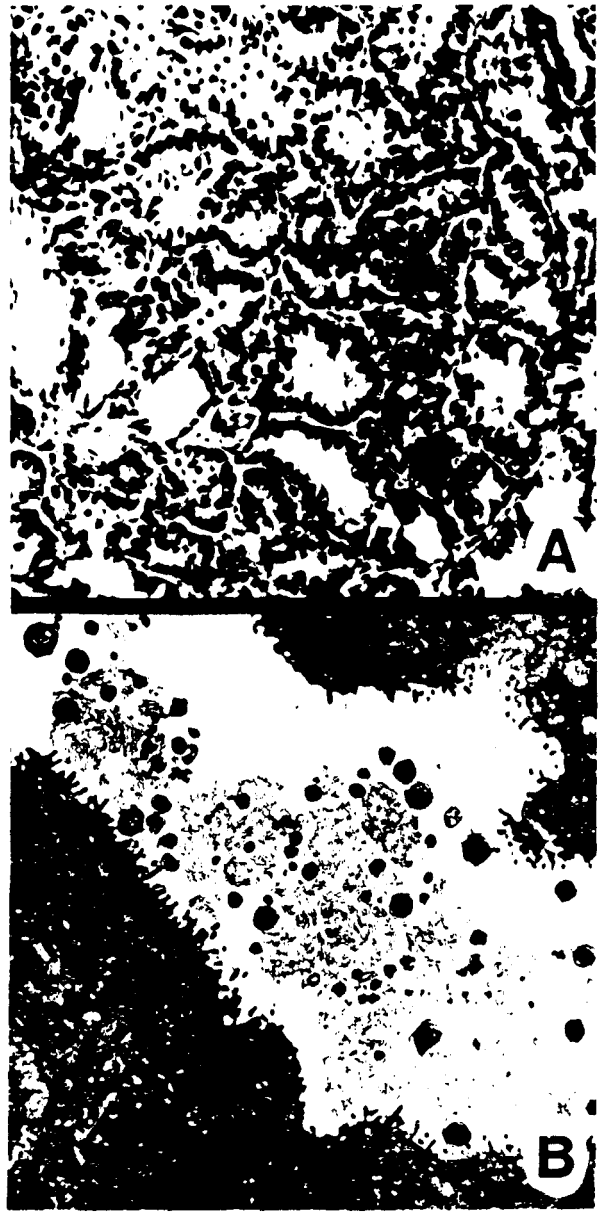

Fig. 5. Photomicrograph and electron micrograph of a papillary adenocarcinoma from a female mouse exposed to 1 ppm of TNM for 2 years. *A*, photomicrograph showing tumor composed of cuboidal to columnar epithelial cells forming irregular glands. H & E. × 150. *B*, electron micrograph of the same tumor showing cells forming a gland have microvilli on their luminal surface and containing developing and mature cytoplasmic lamellar bodies. Tubular myelin (surfactant protein) and lamellar bodies are present in the lumen of the gland. × 6000.

because of inhibitors present in the tumor tissue. Therefore, the rat lung DNA was digested with *Eco*RI for complete digestion. The mutated oligonucleotide allowed detection of a 3.7-kilobase band indicating that the mutated allele was in each of the tumor samples tested whether they were positive (Fig. 4*B*, *Lanes 1-5*, *10* and *11*) or negative (Fig. 4*B*, *Lanes 6-9*) on the transfection assay. The mutated oligonucleotide did not hybridize to the normal rat DNA (Fig. 4*B*, *Lane 12*). Inconsistencies between the transfection data and the oligonucleotide hybridization data may be due to the fact that the K-*ras* is such a large gene and may be difficult to isolate and transfect into the mouse fibroblasts efficiently. The normal allele was also detected in 10 of 11 of the tumors tested (Fig. 4*A*, *Lanes 1-11*) and in normal DNA (Fig. 4*A*, *Lane 12*).

It appears that the tumor DNA examined in Fig. 4 hybridizes to the mutant probe with different levels resulting in variations in the intensities of the bands. This could be due to several reasons. One possibility could be that the mutant probe is hybridizing, although less efficiently, to other mutations in the 12th codon such as those coding for valine (GTT) or alanine (GCT). It must be pointed out, however, that one of those faint bands in Fig. 4*B*, *Lane 11*, is the tumor DNA corresponding to the transfectant DNA in Fig. 3*B*, *Lane 7*. This transfectant DNA was characterized as having a GC→AT transition in the 12th codon. Therefore, it is possible that the faint bands in the rest of the tumor DNA are the result of perfect hybridization of the mutant probe with tumor DNA having the same mutation. It is also unlikely that cross-hybridization occurs because all of these gels were washed above the critical temperature where mismatches should wash off. Another possibility could be differences in the amount of DNA loaded into each well. The most probable cause of the differences in the intensities of bands in the tumor DNA is the difference in the relative amounts of normal DNA compared to the mutant DNA present in a 20-μg sample of tumor DNA.

Activated Oncogenes in TNM-induced Mouse Lung Tumors

A low incidence of spontaneous benign and malignant lung tumors was observed in control mice while mice exposed to TNM had a dramatic dose-related increase of primary lung tumors. As in the rats, there was a dose-related increased incidence, mutiplicity, and frequency of metastasis and invasion of the TNM-induced lung tumors in male and female mice. The earliest observation of a lung tumor was after 54 weeks of treatment in a high dose male. Lung tumors in treated mice were morphologically similar to but larger than those in controls. Morphological features of the tumors were compatible with solid papillary adenomas and adenocarcinomas (Fig. 5*A*) having minimal stromal proliferation. Several of the tumors in treated mice were composed of type II cells with lamellar bodies and, in some instances, tubular myelin was present in glands formed by these cells (Fig. 5*B*). Other tumors had ultrastructural cytological features compatible with Clara cells.

Four of four mouse lung tumor DNA tested induced morphological transformation of the NIH/3T3 mouse fibroblasts after transfection. The transforming frequency ranged from 0.067-0.233 foci/μg DNA. This slightly higher frequency compared to the rat tumor DNA-transforming frequency was probably due to a better quality DNA obtained from the mouse tumors than that obtained from the rat tumors.

The mouse transfectants were then examined for an activated K-*ras* protooncogene. The transfectant DNA and normal mouse lung DNA were digested with *Hind*III and probed with the *Sst*II-*Hinc*III fragment of v-*kis*. Rearranged bands were detected in three of the transfectants (Fig. 6*A*, *Lanes 1-3*), and amplified signals were detected in one of the transfectants (Fig. 6*A*, *Lane 4*) in addition to the background NIH/3T3 DNA bands (Fig. 6*A*, *Lanes 1-4*) and the normal mouse lung DNA bands (Fig. 6*A*, *Lane 5*) at 17.3, 3.6, and 1.6 kilobases. The rearrangements and the amplification of these bands indicated that there was a transfer of the K-*ras* oncogene in these transfectants, similar to that observed in the rat transfectants.

Hybridization of the oligonucleotide probe containing the sequence around the 12th codon with the mutation seen in the

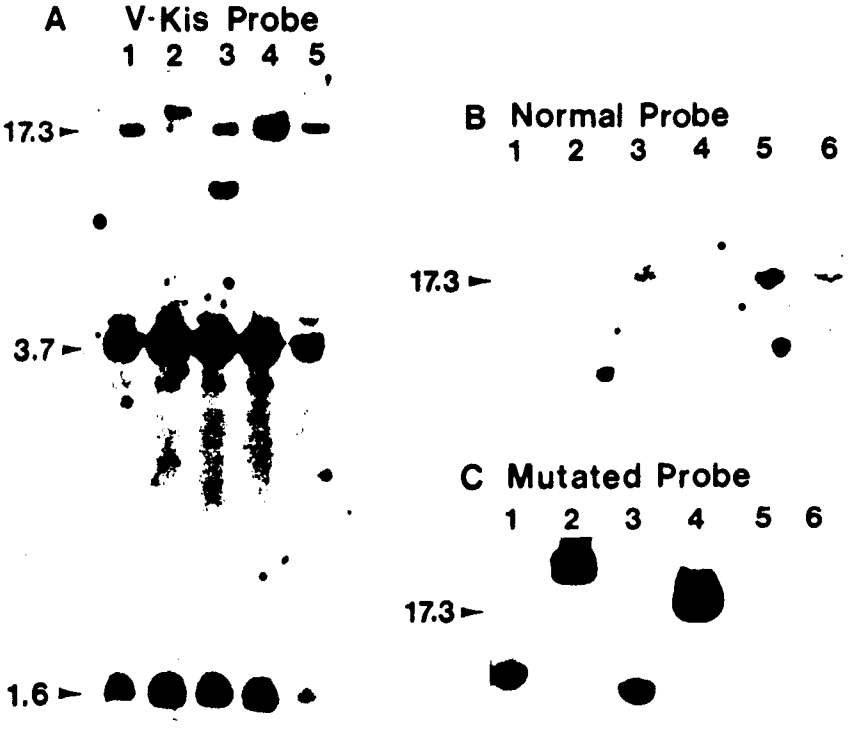

Fig. 6. Activated K-*ras* oncogenes with 12th-codon mutations in TNM mouse transfectants. *A*, detection of rearranged or amplified v-*kis* sequences in *Hind*III-digested TNM mouse transfectants using the HiHi3 v-*kis* probe. *B*, detection of the normal 12th codon of the K-*ras* oncogene in the *Hind*III-digested transfectant DNA (background), in normal lung DNA, and in NIH/3T3 DNA. *C*, detection of a mutated 12th codon in the K-*ras* oncogene of *Hind*III-digested mouse transfectant DNA using the mutated probe described in Fig. 3. In *A*, *B*, and *C*: *Lanes 1-4*, 10 μg four TNM mouse transfectant DNAs; *Lane 5*, 10 μg normal mouse lung DNA. In *B* and *C*: *Lane 6*, 10 μg NIH/3T3 DNA.

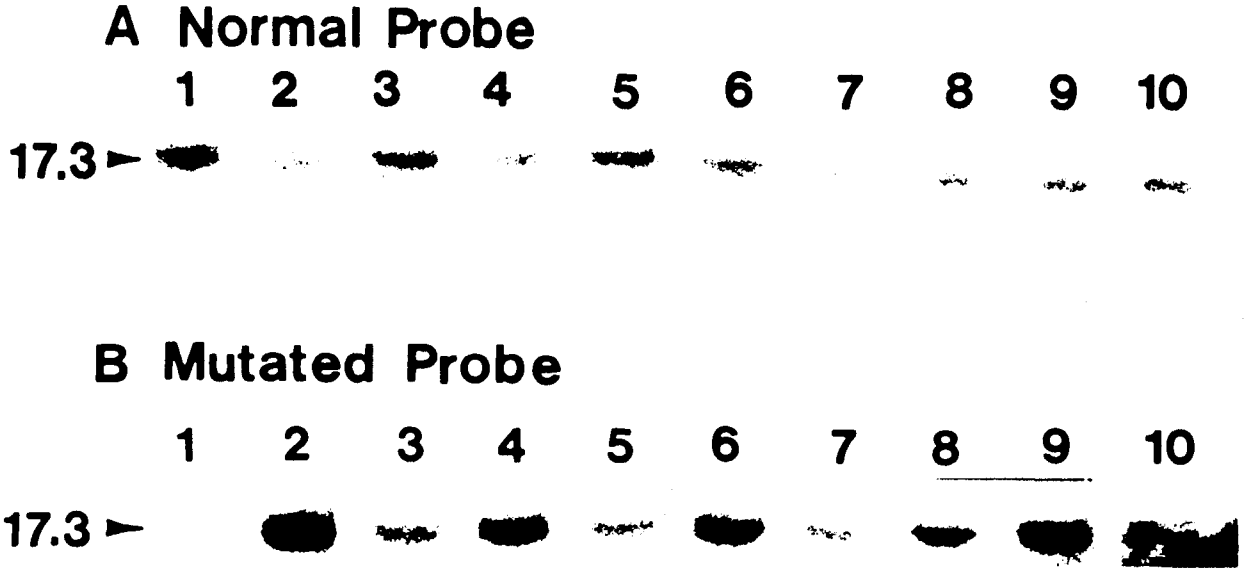

Fig. 7. Detection of a mutated 12th codon in TNM-induced mouse tumors by oligonucleotide hybridization. *A*, detection of the normal allele in the mouse tumor DNA. *B*, detection of the mutated sequence in the mouse tumor DNA using the mutated oligonucleotide probe described in Fig. 3. In *A* and *B*: *Lane 1*, 20 μg of normal mouse lung DNA; *Lanes 2-4* and *8-10*, 20 μg of *Hind*III-digested mouse adenocarcinoma DNA; *Lane 5*, 20 μg of *Hind*III-digested mouse adenoma DNA; and *Lanes 6* and 7, 20 μg of *Hind*III-digested mouse adenocarcinoma/adenoma DNA.

rat DNA indicated that the same mutation was present in each of the mouse transfectants (Fig. 6*C*, *Lanes 1-4*). No hybridization of this probe could be seen with normal mouse lung DNA or NIH/3T3 DNA as expected (Fig. 6*C*, *Lanes 5-6*). The normal probe hybridized to all transfectants indicating the background NIH/3T3 DNA (Fig. 6*B*, *Lanes 1-4*) and to the normal and NIH/3T3 mouse DNA (Fig. 6*B*, *Lanes 5* and *6*).

Examination by oligonucleotide hybridization of nine mouse lung tumor DNA, including the four transfected into NIH/3T3 cells, showed that all of these tumors had the same GC→AT transition as that found in rat TNM-induced lung tumors (Fig. 7*A*, *Lanes 2-10*). These tumors range from benign adenomas (Fig. 7, *Lane 5*) to mixtures of adenomas and carcinomas (Fig. 7, *Lanes 6* and *7*) to carcinomas (Fig. 7, *Lanes 2-4* and *8-10*). Another adenoma (data not shown) also had this GC→AT transition. Each of these tumor DNA also had a normal allele

present that could be detected by oligonucleotide hybridization (Fig. 7*B*, *Lanes 2–10*). As with normal rat DNA, only the normal oligonucleotide would hybridize to normal mouse DNA (Fig. 7, *Lane 1*).

DISCUSSION

This is the first study to show *ras* protooncogene activation in a system where tumors can be induced under conditions similar to human occupational exposure to chemicals. Lung tumors were obtained from two species, the B6C3F$_1$ mouse and the Fischer 344 rat, after long-term chronic exposure to TNM. Histomorphological and ultrastructural features of these tumors are similar to those described for human lung tumors. An activated K-*ras* protooncogene was detected in 100% of the mouse tumors tested and 74% of the rat tumors tested by the NIH/3T3 transfection assay. The detection of the activated K-*ras* gene in two benign mouse tumors suggests that the activation of this gene may be an early event in TNM-induced lung tumors.

The K-*ras* oncogene has been the only oncogene detected by the transfection assay with DNA from human lung tumors and tumor cell lines, with the exception of the HS242 and SW1271 lung tumor cell lines which have activated H-*ras* and N-*ras* oncogenes, respectively (9–13, 23–27). Amplification or increased expression of members of the *myc* oncogene family and the *myb* oncogene has been detected in a number of the human tumors as well (28–32). TNM-induced rodent tumors are the first rodent lung tumors that have been examined for activated oncogenes. Activation of the K-*ras* oncogene in these rat and mouse lung tumors is consistent with the published human lung data. Human and rodent data seem to suggest a tissue-specific activation of a particular protooncogene, at least in the case of the activation of the K-*ras* oncogene in the lung.

A variety of point mutations have been detected in activated *ras* genes from primary tumors and tumor cell lines. At present, K-*ras* protooncogene activation *in vivo* has involved mutations at the 12th codon except in two cases. In one case there is amplification of the normal gene and in another there is a AT→TA transversion in the 61st codon of K-*ras* (4, 6, 9–11, 23–27). The GC→AT transition observed in all of the TNM-induced lung tumors tested may be indicative of a specific lesion in DNA caused by TNM. Point mutations resulting in the activation of protooncogenes in several chemically induced rodent tumors have been consistent with the known alkylation patterns of the carcinogen (1–3, 33–35). For example, mutations at the 12th codon of the H-*ras* detected in rat mammary tumors induced by methylnitrosourea (3) are consistent with the formation of the O^6-methylguanine adduct, and the activating mutation found in DMBA-induced mammary and skin tumors is consistent with DMBA binding to adenosine residues (1, 2, 33, 34).

At present, no information concerning the possible interaction of TNM with DNA is available. However, TNM causes the mutant bacterial strains that detect base pair substitutions TA1535 and TA100 to revert to the wild type by the same GC→AT transition that is observed in the activated K-*ras* oncogene in TNM lung tumors.[4] Since TNM is a known nitrating agent at physiological pH, it could possibly interact with DNA through this mechanism to damage DNA (36). It has also been suggested that nitro-containing compounds may deaminate a base such as cytosine to cause later base mispairing.

[4] E. Zeiger, manuscript in preparation.

Several studies have shown that the loss of the normal allele of oncogenes such as c-H-*ras* and c-*myb* can be correlated with the aggressiveness and/or stage of development of human tumors (37, 38). In this study, we observed that one of 11 rat lung tumors and none of the mouse lung tumors examined had lost the normal allele of the K-*ras* oncogene. A similar loss of the normal N-*ras* allele was seen in a chemically induced thymic lymphoma (39). In that study, Guerrero *et al.* (39) found one tumor with a CG→TA transversion in the 61st codon of N-*ras* and not the normal N-*ras* allele. However, they also found that no tumors were heterozygous in their allelic composition. The presence of the mutated and normal allele in almost all of the TNM-induced lung tumors indicates that the loss of the normal allele is not a prerequisite for tumor formation in these rats and mice. However, this loss could be a sign of aggressiveness or progression as has been suggested by the human tumor data.

Reproducible detection of specific transforming genes in animal model systems strongly suggests that oncogenes play a significant role in the development of these tumors. This is the first study to show that long-term chronic exposure to a chemical is capable of reproducibly activating oncogenes similar to those observed in single dose and initiation-promotion studies. TNM may exert its carcinogenic action by both activation of the K-*ras* oncogene and stimulation of cell proliferation by its irritant properties.

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APPENDIX J

AUDIT SUMMARY

APPENDIX J. AUDIT SUMMARY

The pathology specimens, experimental data, study documents, and draft NTP Technical Report for the 2-year studies of tetranitromethane in rats and mice were audited for the National Institute of Environmental Health Sciences at the National Toxicology Program (NTP) Archives. The audit included review of:

(1) All records concerning animal receipt, quarantine, randomization, and disposition prior to the start of dosing.
(2) All inlife records including protocol, correspondence, animal identification, animal husbandry, environmental conditions, dosing external masses, mortality, and serology.
(3) Body weight and clinical observation data; all data were scanned before individual data for the random 10% sample in each study group were reviewed in detail.
(4) All study chemical records.
(5) All postmortem records for individual animals concerning date of death, disposition code, condition code, tissue accountability, correlation of masses or clinical signs recorded at or near the last inlife observation with gross observations and microscopic diagnoses, consistency of data entry on necropsy record forms, and correlation between gross observations and microscopic diagnoses.
(6) Inventory for wet tissue bags from all animals and residual wet tissues from a random 20% sample of animals in each study group, plus other relevant cases, to evaluate the integrity of individual animal identity and the thoroughness of necropsy and trimming procedure performance.
(7) Blocks and slides of tissues from a random 20% sample of animals from each study group, plus animals with less than complete or correct identification, to examine for proper inventory, labeling, matching of tissue sections, and preservation.
(8) All microscopic diagnoses for a random 10% sample of animals, plus 100% of the changes in diagnoses made to preliminary pathology tables, to verify their incorporation into the final pathology tables.
(9) The extent of correlation between the data, factual information, and procedures for the 2-year studies as presented in the draft Technical Report and the study records available at the NTP Archives.

Procedures and events for the exposure phase of the studies were documented adequately by records at the Archives. Review of the archival records indicated that protocol-specified procedures for animal care were followed adequately. Records that documented the generation, analysis, distribution, and delivery of doses to animals were complete and accurate. Recalculation of the mean body weight values in the Technical Report showed only minor differences in 8/50 values checked.

Data entries on necropsy forms were made appropriately with only minor discrepancies. The date of death recorded at necropsy for each unscheduled-death animal had matching entries among the inlife records for 182/187 rats and 135/139 mice; the date for 1 high dose female rat (carcass ID no. 1191) was transcribed incorrectly (day 356 vs. day 722), and the remaining 8 discrepancies involved differences of 1 to 5 days. Given the overwhelming concentration-related tumor incidences, these relatively minor discrepancies would have no effect on the statistical analyses. The reason for animal removal recorded among the inlife records was in agreement with the disposition code recorded at necropsy for all but 7/600 animals; the overall survival information in the Technical Report reflects corrected mode-of-death data. The condition code for each animal was consistent with the disposition code and gross observations assigned at necropsy.

An individual animal identifier (ear tag) was present and correct in the residual tissue for 63/64 rats and 68/68 mice examined. Review of the entire data trail for the one animal whose ear tag was missing indicated that the integrity of its individual animal identity had been maintained throughout the study. A total of 17 untrimmed potential lesions were found in the wet tissues of 64 rats examined,

and 16 were found in those of 68 mice. Intestinal segments were opened incompletely for 14/64 rats and 8/68 mice examined; however, no untrimmed potential lesions were evident by external examination, and other organs had been opened or incised properly. Each gross observation made at necropsy had a corresponding microscopic diagnosis for all but 16 in rats and 8 in mice; after microscopic review of the slides involved in these noncorrelations, only 2 remained. All slides were present, and tissue sections in corresponding blocks matched properly. All post-Pathology Working Group changes in diagnoses had been incorporated into the final pathology tables. The P values and incidences of neoplasms given in the Technical Report were the same as those in the final pathology tables at the Archives.

In conclusion, examination of the archival records supports the data and results presented in the Technical Report, with the few exceptions indicated above.

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