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TOXICOLOGY AND CARCINOGENESIS STUDIES OF TETRACYCLINE HYDROCHLORIDE

(CAS NO. 64-75-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF TETRACYCLINE HYDROCHLORIDE

(CAS NO. 64-75-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

D. Dietz, Ph.D., Study Scientist

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

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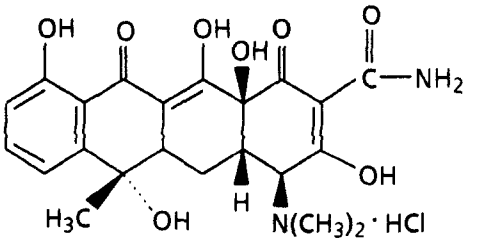
CONTENTS

APPENDIXES



TETRACYCLINE HYDROCHLORIDE

CAS No. 64-75-5

$C_{22}H_{24}N_2O_8 \cdot HCl$ Molecular weight 480.9

Trade names for tetracycline or tetracycline hydrochloride: Achromycin; Amycin; Bristacycline; Cyclopar; Dumocyclin; Neocyclin B; Panmycin; Polycycline; Robitet; Ro-cycline; Steclin; Sumycin; Topicycline; Unimycin

ABSTRACT

Tetracycline hydrochloride is a broad-spectrum antibiotic used for its bactericidal action in human and veterinary medicine. Toxicology and carcinogenesis studies of tetracycline hydrochloride (USP grade, 91% pure) were conducted by feeding diets containing tetracycline hydrochloride to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: The same dietary concentrations were used for the 14-day and 13-week studies (0, 3,125, 6,250, 12,500, 25,000, and 50,000 ppm tetracycline hydrochloride). In the 14-day studies, none of the rats or mice died. The final mean body weight of male rats that received 50,000 ppm was 24% lower than that of the controls. The final mean body weight of mice that received 50,000 ppm in the diet was 18% lower than that of the controls for males and 15% lower for females. No compound-related effects were observed in rats or mice at necropsy.

During the 13-week studies, none of the rats or mice died. The final mean body weight of male rats that received 50,000 ppm was 18% lower than that of the controls. Compound-related effects included cytoplasmic vacuolization in the liver of male rats at 25,000 and 50,000 ppm. Bone tetracycline concentrations in rats and mice increased with increasing dose of tetracycline hydrochloride. The final mean body weight of mice that received 50,000 ppm was 16% lower than that of the controls for males and 6% lower for females. Estimated feed consumption by dosed rat and mouse groups was similar to that of the controls. No compound-related gross or microscopic pathologic effects were observed in mice.

Based on these results, 2-year studies of tetracycline hydrochloride were conducted by feeding diets containing 0, 12,500, or 25,000 ppm tetracycline hydrochloride to groups of 50 rats and 50 mice of each sex for 103 weeks.

Body Weight, Survival, and Feed Consumption in the Two-Year Studies: Mean body weights of dosed and control male and female rats were similar throughout most of the studies. The survival of both the low and high dose female groups was greater than that of the controls. No significant differences in survival were observed between any groups of male rats (male: control, 27/50; low dose, 24/50; high dose, 31/50; female: 27/50; 39/50; 38/50). Mean body weights of dosed mice were markedly (more than

10%) lower than those of the controls throughout most of the studies. The survival rates of the dosed groups of male mice were greater than that of the control group. No significant differences in survival were observed between any groups of female mice (male: 31/50; 43/50; 43/50; female: 37/50; 35/50; 38/50). Feed consumption was similar by dosed and control rats and mice of either sex throughout the studies.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Basophilic cytoplasmic change and clear cell change were positively correlated with tetracycline hydrochloride administration in male rats. Otherwise, no significant increases in neoplastic or nonneoplastic lesions in rats or mice of either sex were considered related to tetracycline hydrochloride administration.

The incidence of adenomas or carcinomas (combined) of the pancreatic islets in low dose male rats was greater than that in the controls (control, 0/49; low dose, 5/49; high dose, 0/49). This marginal effect in the low dose group was not considered to be chemically related. The historical control rate of pancreatic islet cell neoplasms from previous studies at this laboratory is 6% (9/148).

Decreased incidences and severity of chronic nephropathy in male rats were associated with tetracycline hydrochloride administration (48/50; 35/50; 36/50). Female mice administered tetracycline hydrochloride in feed did not develop hepatocellular adenomas or carcinomas (combined incidence: 10/49; 0/48; 0/50). The historical control rate for hepatocellular adenomas or carcinomas (combined) from previous studies at this laboratory is 18/149 (12%). Other decreases in tumor incidence involving several tissues were considered to be of marginal biologic significance.

Genetic Toxicology: Tetracycline hydrochloride was not mutagenic in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, or TA1537) when tested in a preincubation protocol in the presence or absence of exogenous metabolic activation. Tetracycline hydrochloride was negative in the mouse lymphoma L5178Y/TK$^{+/-}$ assay with or without induced rat liver S9 but gave a marginally positive response when tested in the presence of noninduced S9. In cytogenetic assays with Chinese hamster ovary (CHO) cells, treatment with tetracycline hydrochloride, both with and without S9, did not induce chromosomal aberrations or sister chromatid exchanges (SCEs). Tetracycline hydrochloride did not induce sex-linked recessive lethal mutations when administered by feeding or injection to adult male Drosophila.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of tetracycline hydrochloride for male or female F344/N rats and B6C3F$_1$ mice fed diets containing 12,500 or 25,000 ppm. Tetracycline hydrochloride-dosed female rats and male mice had greater survival rates than the respective controls during these studies. Dosed mice had lower body weight than controls, and dosed female mice had no hepatocellular adenomas or carcinomas.

*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.

SUMMARY OF THE TWO-YEAR FEED AND GENETIC TOXICOLOGY STUDIES OF TETRACYCLINE HYDROCHLORIDE

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Dietary concentration			
0, 12,500, or 25,000 ppm tetracycline hydrochloride	0, 12,500, or 25,000 ppm tetracycline hydrochloride	0, 12,500, or 25,000 ppm tetracycline hydrochloride	0, 12,500, or 25,000 ppm tetracycline hydrochloride
Body weights in the 2-year study			
Similar among all groups	Similar among all groups	Lower in dosed groups	Lower in dosed groups
Survival rates in the 2-year study			
27/50; 24/50; 31/50	27/50; 39/50; 38/50	31/50; 43/50; 43/50	37/50; 35/50; 38/50
Nonneoplastic effects			
Reduced incidence and severity of nephropathy in dosed groups (48/50; 35/50; 36/50)	None	None	None
Neoplastic effects			
None	None	None	No liver neoplasms in dosed groups (10/49; 0/48; 0/49)
Level of evidence of carcinogenic activity			
No evidence	No evidence	No evidence	No evidence
Genetic toxicology assays (a)			
Salmonella (reverse gene mutation)	**Mouse L5178Y (Tft resistance)**	**CHO Cells in Vitro**	**Drosophila (sex-linked recessive lethals)**
		SCE / **Aberration**	
Negative/negative	Negative/negative/weakly positive	Negative/negative	Negative/negative	Negative

(a) Responses: −S9/+S9 (induced)/+S9 (noninduced); S9 is liver enzyme fraction for exogenous metabolic activation.

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 Tetracycline Hydrochloride is based on 13-week studies that began in April 1980 and ended in July 1980 and on the 2-year studies that began in January 1981 and ended in February 1983 at Physiological Research Laboratories (Minneapolis, Minnesota).

National Toxicology Program
(Evaluated Experiment, Interpreted Results, and Reported Findings)

D. Dietz, Ph.D., Study Scientist

John Bucher, Ph.D.
Scot L. Eustis, D.V.M., Ph.D.
Joseph K. Haseman, Ph.D.
James Huff, Ph.D.

(Discipline Leaders and Principal Contributors)

Jack Bishop, Ph.D.
C.W. Jameson, Ph.D.
E.E. McConnell, D.V.M.
John Mennear, Ph.D.
G.N. Rao, D.V.M., Ph.D.
B.A. Schwetz, D.V.M., Ph.D.
James K. Selkirk, Ph.D.

NTP Pathology Working Group
(Evaluated Slides and Prepared Pathology Report for Rats on 2/3/87)

Michael Elwell, D.V.M., Ph.D. (Chair) (NTP)
Roger Brown, D.V.M. (Experimental Pathology Laboratories, Inc.)
Olaf Hedstrom, D.V.M., Ph.D. (Oregon State University)
Robert Maronpot, D.V.M. (NTP)
Charles Montgomery, D.V.M. (NTP)
Steven Stefanski, D.V.M. (NTP)
Billy Ward, D.V.M., Ph.D. (Physiological Research Laboratories)

NTP Pathology Working Group
(Evaluated Slides and Prepared Pathology Report for Mice on 2/20/87)

John Seely, D.V.M. (Chair) (PATHCO, Inc.)
James Conroy, D.V.M., Ph.D. (Physiological Research Laboratories)
Scot L. Eustis, D.V.M., Ph.D. (NTP)
Kunitoshi Mitsumori, D.V.M., Ph.D. (NTP)
Suzanne Neuenschwander, D.V.M. Experimental Pathology Laboratories, Inc.
Steven Stefanski, D.V.M. (NTP)
George Szczech, D.V.M., Ph.D. (Burroughs Wellcome Laboratories)

Principal Contributors at Physiological Research Laboratories
(Conducted Studies and Evaluated Tissues)

M. Cowan, Jr., M.S.
B. Ward, D.V.M., Ph.D.
R. Pieper, M.S.
J. Conroy, D.V.M., Ph.D.

Principal Contributors at Experimental Pathology Laboratories, Inc.
(Provided Pathology Quality Assurance)

J. Gauchat
Roger Brown, D.V.M.
Suzanne Neuenschwander, D.V.M.

Principal Contributors at Carltech Associates, Inc.
(Contractor for Technical Report Preparation)

William D. Theriault, Ph.D.
Abigail C. Jacobs, Ph.D.
John Warner, M.S.
Naomi Levy, B.A.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on tetracycline hydrochloride on November 6, 1987, 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.

National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee

Robert A. Scala, Ph.D. (Chair)
Senior Scientific Advisor, Medicine and Environmental Health Department
Research and Environmental Health Division, Exxon Corporation
East Millstone, New Jersey

Michael A. Gallo, Ph.D.*
Associate Professor, Director of Toxicology
Department of Environmental and Community Medicine, UMDNJ - Rutgers Medical School
Piscataway, New Jersey

Frederica Perera, Dr. P.H.
Division of Environmental Sciences
School of Public Health
Columbia University
New York, New York

Ad Hoc Subcommittee Panel of Experts

John Ashby, Ph.D.
Imperial Chemical Industries, PLC
Central Toxicology Laboratory
Alderley Park, England

Charles C. Capen, D.V.M., Ph.D.
Department of Veterinary Pathobiology
Ohio State University
Columbus, Ohio

Vernon M. Chinchilli, Ph.D.*
Department of Biostatistics
Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia

Kim Hooper, Ph.D.
Hazard Evaluation System and Information Services
Department of Health Services
State of California
Berkeley, California

Donald H. Hughes, Ph.D.
Scientific Coordinator, Regulatory Services Division, The Procter and Gamble Company
Cincinnati, Ohio

William Lijinsky, Ph.D. (Principal Reviewer)
Director, Chemical Carcinogenesis
Frederick Cancer Research Facility
Frederick, Maryland

Franklin E. Mirer, Ph.D.
Director, Health and Safety Department
International Union, United Auto Workers, Detroit, Michigan

James A. Popp, D.V.M., Ph.D. (Principal Reviewer) Head, Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, North Carolina

Andrew Sivak, Ph.D. (Principal Reviewer)
Vice President, Biomedical Science
Arthur D. Little, Inc.
Cambridge, Massachusetts

*Unable to attend

SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF TETRACYCLINE HYDROCHLORIDE

On November 6, 1987, the draft Technical Report on the toxicology and carcinogenesis studies of tetracycline hydrochloride received peer 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. D. Dietz, NIEHS/NTP, began the discussion by reviewing the experimental design, results, and proposed conclusions (no evidence of carcinogenic activity for male or female rats, no evidence of carcinogenic activity for male or female mice).

Dr. Sivak, a principal reviewer, agreed with the conclusions He asked for clarification of why reduced incidences of hepatocellular tumors in mice were cited in the Conclusions and significantly reduced incidences of lymphomas (male mice), harderian gland tumors (male mice), and pituitary gland adenomas (male rats) were not. Dr. Dietz explained that the apparently reduced incidences of these other tumors were not cited either because control incidences were high or because decreases in tumor incidences were offset by increases in hyperplasia (pituitary gland). Dr. J. Haseman, NIEHS, added that the negative trend for liver neoplasms was easily the most striking decrease in tumor incidence. Dr. Sivak inquired about the stability of tetracycline hydrochloride in feed in view of the chemical's extreme sensitivity to light. Dr. Dietz said that a 1-week study of tetracycline hydrochloride stability in feed was done under ambient conditions and no breakdown was found. Dr. Sivak thought that it would be useful to show the relationship between the doses used in these studies and the usual human exposures. Dr. Dietz said that the comparisons would be included [see page 58].

Dr. Lijinsky, a second principal reviewer, agreed with the conclusions. Although he considered this study to be a good one, he noted that in female mice, the deficit in weight gain was greater in the first 3 months of the 2-year study than in the 13-week study. He felt that this might suggest a difference in animal treatment or, perhaps, a toxic effect not uncovered in the short-term study.

As a third principal reviewer, Dr. Popp agreed with the conclusions. He questioned the choice of 25,000 ppm as the high dietary concentration for rats when this concentration resulted in liver and bone marrow lesions in the 13-week studies. Dr. Dietz said that the bone marrow atrophy did not seem to be dose related and the liver lesions were not considered to be life threatening and did not serve as a factor in setting the concentrations.

Dr. Sivak moved that the Technical Report on tetracycline hydrochloride be accepted with revisions as discussed and with the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Dr. Lijinsky seconded the motion, which was approved unanimously by the nine members.

I. INTRODUCTION

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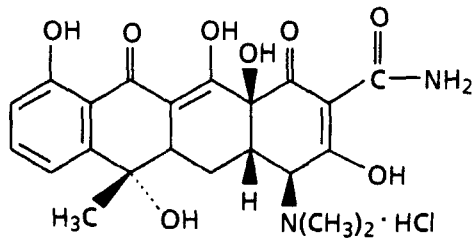

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The tetracyclines as a group refer to a variety of congeners derived from the actinomycetes *Streptomyces aureofaciens* and *S. viridifaciens* or produced semisynthetically from chlortetracycline by reductive dehalogenation (Sande and Mandell, 1985). They possess a wide range of antimicrobial activity against a variety of microorganisms including gram-positive and gram-negative bacteria, rickettsiae, mycoplasma, atypical mycobacteria, and amoebae, but they have very limited effects on fungi. Tetracycline is produced commercially in three forms: tetracycline, tetracycline hydrochloride, or tetracycline phosphate complex (Merck, 1983).

Tetracyclines act by inhibiting protein synthesis (Boothe and Hlavka, 1978; Gale et al., 1981) and are bacteriostatic at lower concentrations and bactericidal at higher concentrations (Siegel, 1978). Although tetracyclines bind to both ribosomes and mRNA, their preferential binding to the 30S ribosomal subunit is the basis for their activity. This binding blocks the enzymatic binding of aminoacyl-tRNA to the ribosomal aminoacyl acceptor site, thereby preventing codon-anticodon interactions. The deleterious lesion to micro-organisms is believed ultimately to involve cell wall synthesis and respiration (Boothe and Hlavka, 1978).

Tetracyclines chelate metal ions, but their chelating potential does not correlate with their antibacterial action (Bowman and Rand, 1980). Some interesting biologic associations, however, have been noted with regard to the ability of tetracycline to bind magnesium. There is evidence that magnesium ions may be involved in the binding of tetracycline to ribosomes rich in magnesium and that chelation of magnesium in bacterial ribosomes precedes the inhibition of protein synthesis (Albert, 1979; Bowman and Rand, 1980), that magnesium ions reduce the ability of tetracycline to bind isolated tRNA (Mikelens and Levinson, 1978), that ingested and other extracellular magnesium ions limit the accumulation of tetracycline into *Bacillus cereus* cells and into the systemic circulation of mammals (Mikelens and Levinson, 1978; Sande and Mandell, 1985), that magnesium ions and tetracycline form liposoluble complexes within bacterial plasma membranes which mediate the transport of tetracycline into the cell cytoplasm (Albert, 1979), and that magnesium ions appear to elicit conformational changes in tetracycline upon binding to the molecule (Albert, 1979).

Isolated ribosomes from both eukaryotic and prokaryotic cells are affected by tetracycline. The selective action of tetracycline on bacteria is dependent on the ability of bacteria and inability of mammalian cells to concentrate these antibiotics (Albert, 1979). Tetracycline transport into bacteria involves an energy-dependent active transport system that pumps tetracycline

through the cytoplasmic membrane. A protein carrier and bivalent cations are postulated to be involved in this transport system (Franklin, 1971; Sande and Mandell, 1985). Bacteria accumulate tetracycline within their cytoplasm by a mechanism whereby the presence of intracellular tetracycline induces a change in the cell membrane permeability. The result is an inhibition of outflow and an unimpaired inflow (Bowman and Rand, 1980). At high concentrations, the tetracyclines permeate mammalian cells and impair protein synthesis (Yeh and Shils, 1966; Tucker and Webster, 1972; Sande and Mandell, 1985). Eukaryotic cells do not take up tetracycline by an active transport system, however.

Physical and Chemical Properties

Tetracyclines are octahydro-naphthacenes and close congeneric derivatives of the polycyclic naphthacenecarboxamide (Albert, 1979; Sande and Mandell, 1985). Their chelating potential is documented in studies of their stability constants, which indicate avidity similar to that of glycine for divalent metallic ions. Tetracycline is an amphoteric drug that exists in cationic form at acidic pH values (less than 3.0), in zwitterionic form at relatively neutral pH values (4.0-7.0), and in anionic form at alkaline pH values (greater than 8.0). The isoelectric pH for tetracycline is about 5.6 (Colaizzi and Klink, 1969; Jaffe et al., 1973). Representative pK_a values are 3.3, 7.7-7.8, and 9.6-9.7 (Colaizzi and Klink, 1969; Albert, 1979). The hydroxyl group at position 3, the dimethylamino group at position 4, and the phenolic β-diketone system occupying positions 10, 11, and 12 change in response to the acid-base balance (Albert, 1979).

Tetracycline hydrochloride is crystallized from hydrochloric acid/butanol. It is stable as a slightly bitter, yellow, crystalline, dry powder in air but darkens on exposure to strong sunlight in moist air and loses activity rapidly when it is in solution or is heated (Merck, 1983; Sande and Mandell, 1985). Solutions of the drug may be stored at room temperature during use, but manufacturers recommend limiting the storage interval between dissolution and use to 12-24 hours (PDR, 1987). Shorter intervals are required for less concentrated solutions.

Tetracycline hydrochloride is moderately hygroscopic and is freely soluble in water (50-100 mg/ml at 22° C); moderately soluble in methanol, ethanol (less than 1 mg/ml at 22° C), and acetone (less than 1 mg/ml at 22° C); slightly soluble in chloroform (2.85 mg/ml at 28° C); and insoluble in ether (Merck, 1983; Keith and Walters, 1985). The pH values recorded from 10 and 20 mg/ml aqueous solutions are 1.8-2.8 and 2.1-2.3, respectively (USP, 1980; Merck, 1983).

Production

Separate production data for tetracycline hydrochloride are not available. Recently reported production data for all tetracyclines, including tetracycline hydrochloride, for all uses are: 1980, 2,977 metric tons (6.5 million pounds); 1981, 3,105 metric tons (6.8 million pounds); 1982, 3,285 metric tons (7.2 million pounds); and 1983, 3,267 metric tons (7.2 million pounds) (CEH, 1982, 1986). In 1979, total worldwide production by fermentation was 9,100 metric tons (20 million pounds). Five of the 23 producers were in the United States (Perlman, 1980). Twenty-four million prescriptions for all tetracyclines were reported for 1985 (FDA, 1986). This number represented 2% of all prescriptions and a 4% decrease from the number of documented prescriptions for 1984.

Use

Tetracycline hydrochloride has a wide variety of therapeutic applications in human and veterinary medicine. In addition, the tetracycline analogs chlortetracycline and oxytetracycline are administered at low levels in livestock feed to prevent disease, promote increased growth, and control bacterial growth in the feed (CFR, 1986). Virtually all boiler poultry and 90% of swine receive some dietary antibacterial supplement, and an estimated 40% or more of all antibiotics manufactured in the United States is used in animal feed (Corbin, 1982). Tetracycline-type antibiotics are approved by the Food and Drug Administration as follows: chlortetracycline in feed at 10-500 g/ton for chickens, mink, swine, sheep, and ducks and as a feed supplement at 0.1-5.0 mg/pound for cattle and 85 mg per day for horses (allowable assay limits are

70%-130% of that specified on the label, and a withdrawal period of 1-15 days before slaughter is required); oxytetracycline in feed at 50-500 g/ton for chickens and turkeys and as a feed supplement at 75-80 mg per day for cattle and 250 mg/kg per day for fish (allowable assay limits are 65%-135% of that specified on the label, and a withdrawal period of 1-7 days before slaughter is required). The tolerance limit for tetracycline residue levels in uncooked meats is 0.25 ppm (CFR, 1986).

Tetracycline hydrochloride is administered by oral, parenteral, dermal, and ocular routes. It is effective against gram-positive and gram-negative bacteria, rickettsiae, mycoplasma, chlamydia, atypical mycobacteria, and amoebae but has little activity by itself against true fungi. Even though gram-positive micro-organisms are affected by tetracycline at lower concentrations than are gram-negative species, gram-positive species are more likely to develop a resistance to tetracycline (Sande and Mandell, 1985). Tetracycline has been cited as the drug of first choice for treating chlamydia and rickettsial infections, which include Rocky Mountain spotted fever and typhus (Shadomy and Mayhall, 1979; Sande and Mandell, 1985). Tetracycline hydrochloride is also reported to be effective against acne because of its ability to decrease the fatty acid content of sebum (Kraus, 1968; Sande and Mandell, 1985). Topical and oral forms have been used to treat acne, but local application of tetracycline to skin is contraindicated when hypersensitivity is possible (Sauer, 1976; Hubbell et al., 1982; Thibodeau and Robert, 1982; Wong et al., 1984; Sande and Mandell, 1985; Gammon et al., 1986; PDR, 1987). Allergic reactions may also occur after oral administration of tetracycline (Sande and Mandell, 1985). The topical application of tetracycline hydrochloride as an ophthalmic ointment or suspension is not generally contraindicated even when hypersensitive reactions are possible.

The following are typical doses of tetracycline hydrochloride prescribed for humans (Sande and Mandell, 1985; PDR, 1987):

Oral forms--1-2 g divided into two to four equal doses. Administered 1 hour before or 2 hours after a meal. Formulations are available as capsules, tablets, flavored powders, suspensions, and drops and as syrups or elixirs for pediatric use.

Intramuscular--250 mg every 24 hours or 300 mg given in divided doses at 8- to 12-hour intervals to adults.

Intravenous--250-500 mg every 12 hours.

Ophthalmic--0.5%-3% ointment or 1% suspension. The medication is prepared fresh every 7 days and kept refrigerated. One or two drops are instilled into the conjunctival sac every 2 hours.

Dermal--2.9 mg in solution is administered twice daily to the face and neck, or 4.8 mg in solution is administered twice daily to other areas of the skin.

Veterinary therapeutic uses of tetracycline hydrochloride include the prevention and treatment of gram-positive and gram-negative bacterial and mycoplasmal infections. The drug is administered primarily by the oral route to a variety of species (Veterinary Pharmaceuticals & Biologicals, 1983).

Absorption, Distribution, and Excretion

Tetracycline hydrochloride is incompletely absorbed from the gastrointestinal tract, with approximately 60%-80% absorbed from an empty stomach (Sande and Mandell, 1985). Green et al. (1976) reported that approximately 80% of orally administered tetracycline is absorbed in humans, whereas Kelly and Buyske (1960) reported lower oral absorption of tetracycline by rats and dogs than by humans. Incomplete gastric absorption may occur because tetracyclines are ionized under the acidic and basic conditions within the gastrointestinal tract (Schanker, 1971). Tetracyclines are absorbed primarily by passive diffusion in a lipid-soluble neutral state at the isoelectric point corresponding to a pH of approximately 5.6 (Schanker, 1971; Jaffe et al., 1973). Optimal pH values for absorption are found in the stomach and upper small intestine, where maximal absorption occurs (Sande and Mandell, 1985). Barr et al. (1971) showed that sodium bicarbonate ingestion decreased tetracycline absorption in humans by 50% and attributed this effect to a decreased dissolution of

tetracycline. Other factors affecting oral absorption include the administered dose, exposure to other drugs, and competing elements in the diet (Sande and Mandell, 1985). The percentage of drug absorbed is greater at lower doses and in fasted animals.

Insoluble drug-cation complexes form with aluminum, iron, calcium, and magnesium and thereby prevent gastrointestinal absorption of tetracyclines. The concomitant administration of food, milk, and dairy products containing calcium, antacids containing magnesium or aluminum, and iron preparations are therefore contraindicated during tetracycline treatment. Exposure to these interfering materials should be discontinued 1 hour before and not resumed until 1 hour after drug administration. A corollary to these observations is that prolonged exposure to tetracycline may result in significantly reduced gastric absorption of essential cationic elements such as iron (Neuvonen et al., 1975). Phosphate ion improves tetracycline absorption in part by removing calcium (Sande and Mandell, 1985).

Approximately 20%-60% of absorbed tetracycline becomes bound to plasma proteins, primarily in the albumin fraction (Kunin et al., 1959; Bernheim, 1971; Goldstein et al., 1974; Green et al., 1976; Harvey, 1980; Sande and Mandell, 1985). Plasma protein binding can significantly influence the drug delivery rate to metabolic, receptor, and elimination sites and thereby affect the total body clearance of drugs (Raghuram and Krishnaswamy, 1981). The percentage of plasma protein bound to tetracycline in dogs (36%) was comparable to that measured in humans (32%) (Wozniak, 1960). The plasma half-life after a single dose of tetracycline is reported to be 8.5 hours in humans (Kunin et al., 1959; Bowman and Rand, 1980). Peak plasma concentrations are attained within 2-4 hours after a single oral dose of tetracycline and decrease slowly over the next 12-24 hours (Bernheim, 1971; Sande and Mandell, 1985). Tetracycline administration every 6 hours is recommended to attain relatively constant therapeutic plasma concentrations. Oral doses of 250 and 500 mg tetracycline every 6 hours to humans result in mean plasma levels of 3 and 4.5 µg/ml (Bernheim, 1971; Sande and Mandell, 1985) which are above the therapeutic plasma tetracycline concentrations (1-3 µg/ml) indicated by Noble et al. (1967), who measured 2.2 and 3.0 µg/ml tetracycline in rat plasma 3 hours after the administration of single oral doses of 25 and 100 mg/kg, respectively. Greenberger et al. (1967) administered 400 mg/kg tetracycline to rats by a single intraperitoneal injection and determined serum levels after 2, 4, and 8 hours to be 171, 122, and 81 µg/ml, respectively. Breen et al. (1975) gave male and female rats single intravenous doses of tetracycline (50-200 mg/kg) and, after 3 hours, measured tetracycline concentrations of 16.8-64.8 µg/ml in the serum and 164-664 µg/g in the liver. These concentrations were dose related. The volume of distribution for tetracyclines in humans is reported to range from 0.75 to 1.89 ml/g (Harvey, 1980).

Tetracycline kinetics in humans administered a single dose is described by a two-compartment open model (Raghuram and Krishnaswamy, 1981). The decline in plasma tetracycline concentration after a single exposure is represented by an initial rapid α distribution phase followed by a slower β elimination phase. The half-lives for the α and β phases are reported to be 0.51 and 10.55 hours, respectively. Undernourished subjects with depressed levels of serum albumin exhibit a more rapid total body clearance, with α and β half-lives of 0.32 and 5.40 hours, respectively. Plasma half-lives of tetracycline tend to increase in older subjects (Richey and Bender, 1977) whose metabolic and excretory functions are declining.

Within the systemic circulation, tetracycline is widely distributed throughout the body fluids and tissues and is concentrated in the reticuloendothelial cells of the liver, spleen, and bone marrow and in bone, kidney, intestinal mucosa, muscle, and the dentine and enamel of unerupted teeth (Greenberger et al., 1967; Boothe and Hlavka, 1978; Sande and Mandell, 1985). In the liver, the drug undergoes enterohepatic circulation whereby it is excreted via the bile into the intestine and mostly reabsorbed. Biliary concentrations of tetracycline are reported to be 5-10 times higher than plasma values. Breen et al. (1975) perfused rat livers with tetracycline for 4 hours and evaluated bile for tetracycline content. Groups of rat livers were perfused with

2.5, 5.0, 10.0, or 20.0 mg tetracycline; the respective bile concentrations were 96.7, 100.1, 150.2, and 205 µg tetracycline/ml bile, which represented 6.4%, 3.8%, 2.4%, and 0.9% of the administered tetracycline. Enterohepatic circulation delays elimination of tetracycline from the blood and is responsible for its detection in the intestine and feces after parenteral administration.

There is little penetration of tetracycline into cerebrospinal fluid (CSF) after oral administration. CSF concentrations are normally 2%-10% those in the plasma (Harvey, 1980). By contrast, tetracycline readily crosses the placenta and enters the fetal circulation and amniotic fluid within 2 hours of maternal exposure (Asling and Way, 1971; Ginsburg, 1971). Concentrations of the drug in fetal plasma, umbilical cord plasma, and amniotic fluid are reported to reach 70%, 60%, and 20% of maternal plasma levels, respectively (Asling and Way, 1971; Sande and Mandell, 1985). Rall et al. (1957) showed that tetracyclines concentrate and persist in implanted tumor tissues of rats and mice. The ability of tetracycline to localize and remain in tumor tissue has been used as a diagnostic tool for differentiating malignant from benign lesions in gastric tissue, mammary tissue, and bone, where tetracycline may attain levels twice those noted in adjacent healthy tissue (Bernheim, 1971; Boothe and Hlavka, 1978). When their administration is discontinued, the tetracyclines are eliminated rapidly from all tissues except those of tumors, bone, and teeth (Fabre et al., 1971).

The primary route of tetracycline excretion is in the urine; 20%-60% is excreted within 24 hours after an intravenous dose of 0.5 g tetracycline or any oral dose (Sande and Mandell, 1985). Alkalinization of human urine enhances the cumulative renal clearance of tetracycline (Jaffe et al., 1973; Ylitalo et al., 1977) because the renal tubular epithelium is selectively permeable to lipid-soluble, un-ionized drugs. The highest percentage of lipid-soluble tetracycline species (zwitterionic form) occurs at the isoelectric pH of approximately 5.6 (98.7%). Significant concentrations of the zwitterionic species are also present at pH values ranging from 3.9 (78.8%) to 6.6 (93.3%).

Urinary pH may be a more important consideration for human than for rodent (rat) renal clearance because of the greater range in urinary pH values for normal humans (4.8-7.8) compared with those for laboratory rats (7.3-8.5) (Mitruka and Rawnsley, 1981). Since renal function plays an important role in tetracycline excretion, patients with preexisting renal impairment are administered reduced doses of the drug to prevent toxic side effects (Bernheim, 1971; Sande and Mandell, 1985). Patients with severe renal disease may exhibit a plasma tetracycline half-life of 4-5 days (Eisner and Wulf, 1963; Fabre et al., 1971). Significant tetracycline excretion also occurs in the feces because of incomplete absorption after oral doses and enterohepatic circulation after any route of administration. Eisner and Wulf (1963) administered radiolabeled tetracycline intravenously to rats and collected urine and feces samples for 72 hours after drug administration. In their studies, 69.2% and 19.5% of the radioactivity was recovered in the urine and feces, respectively. The feces/urine ratio (0.28) indicated comparatively less fecal elimination of tetracycline than of the three other tetracycline analogs studied. Noble et al. (1967) collected urine samples from rats for 48 hours after single oral doses of tetracycline at 25 or 100 mg/kg and reported that 7.5% or 1.6% of the administered dose was excreted in urine.

Relatively high concentrations of tetracyclines are also secreted in milk (Sande and Mandell, 1985). Bovine and human milk/plasma ratios of 1.6 and 0.62-0.81, respectively, have been reported (Plaa, 1971; Welch and Findlay, 1981).

Tetracycline does not appear to undergo metabolic transformation in humans (Fabre et al., 1971), rats, or dogs (Eisner and Wulf, 1963).

Short-Term Toxicity

LD_{50} values for tetracycline hydrochloride are reported to be 128, 300, and 700 mg/kg by the intravenous, intraperitoneal, and subcutaneous routes in rats and 3.58 g/kg after oral exposure in mice (Greenberger et al., 1967; NIOSH, 1984). Wivagg et al. (1976) determined LD_{50} values in mice after intraperitoneal and subcutaneous administration of tetracycline hydrochloride.

Their results showed that formulations prepared at the isoelectric pH (5.6) produced lower LD_{50} values that were attributable to greater lipid solubility and enhanced absorption of the zwitterion.

Large doses of tetracycline have been shown to induce hepatic dysfunction in rats (Seto and Lepper, 1954; Lewis et al., 1967; Zussman, 1968; Damjanov and Solter, 1970) and humans (Pflug, 1963; Dowling and Lepper, 1964; Wruble and Cummins, 1965), as indicated by fatty degeneration, jaundice, azotemia, bilirubinemia, increased serum glutamic-oxaloacetic transaminase and alkaline phosphatase, focal venous tromboses and thrombosed central veins, histochemical alterations of succinic dehydrogenase and alkaline phosphatase, and increased activity of the urea cycle liver enzyme, ornithine carbamoyl transferase. Lepper et al. (1951) observed hepatic changes (lipid vacuolation) in mice exposed to 75-150 mg/kg chlortetracycline hydrochloride and 250 mg/kg oxytetracycline hydrochloride. Gray et al. (1974) noted fatty changes in the liver of rats receiving 100 mg/kg tetracycline hydrochloride by intraperitoneal injection. Breen et al. (1975) administered 50-200 mg/kg tetracycline hydrochloride intravenously to rats and reported increased levels of hepatic triglycerides 3 hours later in males and females receiving 100 mg/kg or more of the drug. Livers removed from rats 3 hours after tetracycline administration and perfused with oleic acid had increased hepatic triglyceride content in males receiving at least 50 mg/kg tetracycline and in females receiving at least 75 mg/kg tetracycline.

Other in vitro studies with mouse and rat liver mitochondria showed that tetracycline inhibits oxidative phosphorylation (Du Buy and Showacre, 1961; De Jonge, 1973; Shapiro et al., 1977). Deosthale and Tulpule (1969) administered tetracycline to rats at 10 mg per rat per day for 15 days and recorded increased activity of ornithine carbamoyl transferase in addition to increased urinary urea nitrogen and blood ammonia nitrogen levels. Noble et al. (1967) recorded increased serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase activity indicative of liver toxicity in a dog that received an intravenous dose of 40 mg tetracycline/kg body weight. Parenteral doses of at least 2 g per day are required to cause hepatic injury in humans (Dowling and Lepper, 1964; Wruble and Cummins, 1965; Bernheim, 1971; Sande and Mandell, 1985). A proposed mechanism for tetracycline-induced hepatotoxicity is related to the ability of this drug to localize in the mitochondria and interfere with oxidative phosphorylation (Du Buy and Showacre, 1961; Lewis et al., 1967; Damjanov and Solter, 1970; Gray et al., 1974). Depressed ATP levels or changes in ATP associated with this tetracycline-mediated change could possibly disrupt fatty acid oxidation that requires ATP. Alternative and more widely accepted proposed mechanisms involve the ability of tetracycline to inhibit lipoprotein synthesis that is required for the outflow of hepatic triglyceride or the ability of this drug to complex to preexisting lipoprotein and thereby inhibit hepatic triglyceride release (Damjanov and Solter, 1970; Breen et al., 1972; Gray et al., 1974).

Preexisting renal insufficiency may be exacerbated by tetracycline therapy. In addition, a condition known as Fanconi syndrome, characterized by nausea, vomiting, polyuria, polydipsia, proteinuria, acidosis, glucosuria, and gross amino aciduria, can occur in patients ingesting outdated and degraded tetracycline containing anhydro-4-epitetracycline (Bernheim, 1971; Sande and Mandell, 1985). These renal changes are associated with metabolic alterations involving the antianabolic ability of tetracycline to inhibit protein synthesis and may also be associated with weight loss, anorexia, hypovolemia, and azotemia. Rosenberg and Wahlstrom (1974) coadministered tetracycline (75 mg/kg per day for 4 days) by intraperitoneal injection and methoxyflurane (1% in air for 45 minutes on day 2) to rats and noted shrinkage of the glomeruli and increased protein deposits in the tubules of the kidney compared with rats that received only the anesthetic.

In another study, rats receiving a single intraperitoneal dose (400 mg/kg) of tetracycline showed increased blood urea nitrogen values, hyperkalemia, and acidosis 4 and 8 hours after drug exposure (Greenberger et al., 1967). Kidneys from rats administered oxytetracycline at 100 mg/kg for 10 days were pale and swollen upon gross examination and showed

inflammatory changes upon histologic examination (Tarara et al., 1976). Two dogs receiving tetracycline hydrochloride intravenously at 40 mg/kg per day in divided doses for 1 month exhibited polydipsia (characterized by approximately double their predosing intake of water) and polyuria when they were evaluated on days 19-26 (Noble et al., 1967). The diuresis is probably secondary to the tetracycline inhibition of protein synthesis. The resulting negative nitrogen balance with rising serum levels of nonprotein nitrogen increases the blood volume and urine output because of an increase in osmotically active elements (Steiner et al., 1965).

The most common side effects associated with oral use of tetracycline by humans are gastrointestinal, manifested by epigastric burning, nausea, vomiting, and diarrhea (Bernheim, 1971; Sande and Mandell, 1985). These symptoms may be related to changes in the intestinal flora as well as to direct irritation by tetracycline of the gastrointestinal mucosa. Wistar rats injected intramuscularly with oxytetracycline (300 mg/kg) for 3 days or longer showed severe damage to the structure and function of the small intestine epithelium (De Jonge, 1973).

Tetracycline has a high affinity for active sites of calcification in the developing teeth and bones of fetuses, neonates, and infants who are particularly susceptible to the demineralizing effects of this drug (Bernheim, 1971; Sande and Mandell, 1985). Deposition of the drug in teeth and bones may be related to its chelating property, resulting in the formation of a tetracycline calcium orthophosphate complex or a more complex reaction involving collagen fibers. A diagnostic sign of this effect is a characteristic yellow fluorescence of teeth and bones, resulting from the accumulation of a pigment that has an ultraviolet absorption maximum at 270 nm. After continued treatment, the yellow fluorescence is replaced by a nonfluorescent brown pigment that may represent an oxidation product of the antibiotic. A few isolated occurrences involving an association between tetracycline exposure and hyperpigmentation include rare, documented cases of brown pigment in the thyroid gland (White and Besanceney, 1983) and yellow pigment in the fingernails (Hendricks,1980) of patients receiving tetracycline. The effects on developing teeth of exposure to tetracycline are enamel and tooth crown discoloration (Kerley and Kollar, 1978; Sande and Mandell, 1985).

Several studies have described tetracycline's deleterious effects on in vitro bone development and have attempted to describe the underlying mechanism(s). Saxen (1965, 1966a,b) exposed organotypic cultures of embryonic bones (ulnar and radial bone rudiments) from 14- to 17-day-old mouse embryos to tetracycline at 0.01-100 µg/ml. Tetracycline at doses of 1 µg/ml and higher depressed the normal increase in bone calcium, shortened the calcified zone, and decreased the calcium content of previously mineralized bone. Tetracycline (10 µg/ml) depressed [H^3]thymidine incorporation into DNA. These inhibitory effects were reversible after short-term exposure but not after prolonged exposure. The investigations indicate that low doses affecting in vitro mineralization do not induce any changes in the synthesis of the organic matrix.

By contrast, only a few in vivo studies describing results of tetracycline administration to mammals have reported adverse effects on growth and development of bone and teeth; cosmetic discoloration has been noted. In addition, the interpretation of results from these studies is confusing because of the high doses necessary to produce adverse effects and the absence of corroborating evidence from other investigators. Maternal exposure to tetracycline at 100 mg/kg administered by intraperitoneal injection resulted in minor skeletal malformations and inhibition of skeletal growth in mouse fetuses and neonates (Kaitila et al., 1970; Mahaney, 1982); depressions in the growth rate of long bones were observed in premature children who received high doses of tetracycline (Kaitila et al., 1970). Johnson and Mitchell (1966) dosed female rats with oral pediatric drops of tetracycline (3-20 mg/pound body weight) from mating until parturition and killed the pups 21 days after birth. The teeth and bones were not visibly affected (i.e., the teeth were not hypoplastic or discolored), and femur lengths were normal with no hypocalcification noted in bone-section historadiograms. The only positive finding was a yellow fluorescence that was noted in the teeth and bones under ultraviolet light.

The combined data from in vitro and in vivo studies indicate that high doses (30 µg/ml) of tetracycline have a depressive effect on the nucleation phase, involving collagen biosynthesis, of tooth and bone development, whereas low doses (5 µg/ml) inhibit mineralization or the crystal growth phase (Halme et al., 1969; Kaitila et al., 1970; Saxen and Kaitila, 1972). Mineralization or calcification and collagen biosynthesis in bones often occur as parallel events during osteogenesis, and the effect of tetracycline on both these components of bone development has been described extensively (Halme et al., 1969; Kaitila et al., 1970). The protein collagen, which accounts for about 95% of the organic bone matrix, contains about 14% hydroxyproline, an amino acid found exclusively in collagen (Halme and Aer, 1968; Halme et al., 1969; Kaitila et al., 1970). Hydroxyproline is biosynthesized in the polypeptide precursor of collagen (protocollagen) by the hydroxylation of proline. Tetracyline inhibits the uptake of radiolabeled proline by cultured bone and thus inhibits the enzymatic conversion of proline to hydroxyproline. The ability of tetracycline to chelate ferrous ion, an essential cofactor of protocollagen hydroxylase, also is suggested as the mechanism for this effect. As a potent chelator, tetracycline is thought to act directly by binding divalent cations and thus either interferes directly with the formation of bone mineral crystals or removes cations participating in an enzymatic process involved in crystallization. Shapiro et al. (1977) showed that tetracycline interferes with the intramitochondrial accumulation of calcium, which is reported to be the first step in the calcification process.

The clinical literature describes various hypersensitivity reactions resulting from administration of tetracycline, including skin rash, anaphylaxis, eye irritation, fever, and eosinophilia (Bernheim, 1971; Sande and Mandell, 1985). Experiments designed to test the effects of tetracycline on immune function generally indicate that tetracyclines at higher than therapeutic doses are immunosuppressive. Klimova et al. (1979) suggested that the epimers and anhydro derivatives of tetracycline (formed during storage) are 40-100 times more immunodepressive than is tetracycline. In vitro administration of tetracycline depressed the ability of human leukocytes to destroy yeast and bacteria by phagocytosis; leukocytes from healthy humans who ingested tetracycline demonstrated a decreased phagocytic capacity for yeast (Forsgren et al., 1974). Tetracycline can inhibit chemotaxis of human polymorphonuclear neutrophils at concentrations (25-100 µg/ml) above the therapeutic level (Majeski and Alexander, 1977; Forsgren and Schmeling, 1977; Forsgren et al., 1978). This effect is believed to be mediated by tetracycline inhibition of protein synthesis. The third observation is the decreased in vitro mitogenic response of tetracycline-treated human T-lymphocytes to phytohemagglutinin compared with that of untreated lymphocytes (Munster et al., 1977; Forsgren and Banck, 1978; Banck and Forsgren, 1979). Tetracycline at concentrations exceeding human therapeutic levels is required to elicit this effect, however. Tetracycline at high concentrations (0.1 mg/ml) reverses the hemagglutination reaction to *Escherichia coli* (Roland et al., 1980).

Phototoxic side effects reported in conjunction with the clinical use of tetracycline in humans include photosensitization reactions characterized by photo-onycholysis (separation of the nail plate from the nail bed at its distal and lateral attachments) and porphyrialike and sunburnlike cutaneous changes (Bernheim, 1971; Epstein et al., 1976; Lasser and Steiner, 1978; Kanwar and Singh, 1979; Ibsen and Andersen, 1983; Sande and Mandell, 1985). Combined exposure to ultraviolet light in the range 270-320 nm (sunlight) and to tetracycline is recognized as inducing these conditions.

Because of the inherent irritative property of tetracycline, intramuscular injection of the drug produces severe pain if it is not coadministered with a local anesthetic (Sande and Mandell, 1985).

Long-Term Toxicity and Carcinogenicity

Deichmann et al. (1964) fed diets containing tetracycline to male Osborne-Mendel rats and male dogs for 2 years. The studies in rats included 180 controls, and 100 rats received 100 ppm tetracycline, 130 rats received 1,000 ppm tetracycline, and 100 rats received 3,000 ppm tetracycline. Ten rats from the control and 1,000-ppm groups were killed after 3, 6, 9, 12,

15, and 18 months, and 10 rats from the 100- and 3,000-ppm groups were killed after 12, 15, and 18 months. The dosed groups appeared to be more vigorous and healthy and showed a more rapid gain in body weight during the initial 18 months than did the controls. Feed consumption correlated positively with body weight gain. The percentage of survivors during the studies for the control, 100-, 1000-, and 3,000-ppm groups was 57%, 60%, 72%, and 70%, respectively. Pneumonia occurred at lower incidences in all tetracycline-dosed groups than in controls; nephropathy and pale kidneys were more frequent in rats dosed with 3,000 ppm tetracycline. Nontoxic effects included bilateral, slight-to-moderate brownish pigmentation in the thyroid gland (all dose groups) and a yellow pigmentation of the long bones and calvarium (3,000-ppm group only).

Although the rat studies by Deichmann et al. (1964) generated useful information, they did not provide an adequate data base to evaluate the long-term toxicity or carcinogenicity of tetracycline because of the use of one sex (male) and the high incidence of pulmonary infection (pneumonia) in control (34%) and dosed (11%-28%) groups.

The studies with dogs included eight male dogs per group administered 0, 1,000, 3,000, or 10,000 ppm tetracycline in the diet (Deichmann et al., 1964). No adverse or beneficial effects were associated with exposure to tetracycline. The only changes observed were a yellowish coloration of the bony structures (calvarium, ribs, and femurs) and the presence of brown granules in the cytoplasm of thyroid follicular epithelial cells (all dosed dogs).

Dessau and Sullivan (1961) fed diets containing chlortetracycline to male and female Sherman rats for 2 years at concentrations of 0, 1, 5, 20, 100, 500, 2,000, 10,000, and 50,000 ppm. Results from those studies show that survival for males and females fed diets containing 500, 2,000, or 10,000 ppm chlortetracycline and females fed diets containing 50,000 ppm chlortetracycline was appreciably greater than for controls. Clinical signs noted in the 50,000-ppm groups included encrustations about the nose and mouth, increased salivation (reflecting the irritant properties of chlortetracycline), and abdominal distension (a sign of disturbed gastrointestinal function). Males and females consuming the 50,000-ppm diet gained weight at a slower rate and attained a lower maximum weight than did controls. The leukocyte counts were depressed in the male and female 50,000-ppm groups, all dosed males showed depressed neutrophil counts, and all groups consuming 500 ppm or more chlortetracycline showed depressed lymphocyte counts compared with those for controls. Bones from rats consuming 500 ppm chlortetracycline or more were yellow and fluoresced under ultraviolet light. Middle ear and lung infections were reduced in rats receiving diets containing 500 ppm or more chlortetracycline. Several male rats from the 50,000-ppm group died early in the studies; these animals were emaciated, the internal organs were atrophied, and the livers were fatty. A foreign body reaction characterized by the presence of monocytes and multinucleated giant cells and occasional interstitial fibrosis was a frequent observation in the 50,000-ppm group. This effect was attributed to the irritant effect of dust inhaled from the diet. Finally, the incidence of pituitary gland neoplasms was lower in males and females from the 50,000-ppm group than in controls, and the incidence of mammary neoplasms was lower in the 50,000-ppm females than in controls.

Two-year studies of oxytetracycline were conducted by feeding rats diets containing 0, 25,000, or 50,000 ppm oxytetracycline hydrochloride; mice received diets containing 0, 6,300, or 12,500 ppm oxytetracycline hydrochloride (NTP, 1987). Mean body weights for high dose rats and high dose male mice were 5%-8% lower than those for controls, and the survival rate for control male rats was lower than that for the high dose group. The evidence for carcinogenicity was equivocal based on a marginal increase in pheochromocytomas of the adrenal gland in male rats and on adenomas of the pituitary gland in female rats. There was no evidence of carcinogenicity of oxytetracycline in mice.

Reproductive Effects and Teratogenicity

Ravid and Toaff (1972) indicated that tetracyclines may be administered safely to women in early pregnancy but are contraindicated after week 25.

Tetracycline's effects on bone mineralization and coloration during the developmental and neonatal periods are reported in the short-term toxicity section of this Introduction. In addition to these effects, minor skeletal anomalies have been reported in the offspring of rats and mice dosed orally with 250 mg/kg tetracycline from gestational day 5 to day 20 (Filippi and Mela, 1957; Bevelander and Cohlan, 1962). Other investigators, however, did not observe limb malformations in the offspring of pregnant rats administered 150-500 mg/kg tetracycline (McColl et al., 1965). Steiner et al. (1965) administered tetracycline daily by intraperitoneal injection to pregnant rats beginning on gestational day 14 at a level of 85 mg/kg for 2-5 days. They noted a compound-related increase in abortions and stillbirths and a decrease in fetal weight in the absence of malformations and attributed their findings to a direct toxic effect of tetracycline on the trophoblast cells of the labyrinth and basal placental areas. Degenerative placental changes were recorded in 17%, 60%, and 75% of the rats dosed for 2, 3-4, and 5 days, respectively.

Boucher (1969) noted that chlortetracycline administered at 125 mg/kg per day by subcutaneous injection to pregnant mice interrupted gestation by disturbing nidation. Administration of progesterone, progesterone plus estradiol, or prolactin reestablished gestation in these mice. Bevelander and Cohlan (1962) injected pregnant Wistar rats intramuscularly with 40-80 mg/kg per day tetracycline in daily divided doses (8 hours apart) during gestational days 12-20, 8-15, or 10-15. Rats were killed on gestational day 20, and fetuses were removed and examined. Exposure to tetracycline on gestational days 10-15 and 8-15 resulted in a 28% reduction in expected fetal body weight, reduction in fetal size, and a marked secondary yellow fluorescence of the skeleton. No gross malformations or anomalies were recorded.

McColl et al. (1965) administered tetracycline (500 mg/kg per day) in feed to female rats from 3 days before mating until delivery by section on gestational day 21. They noted an increased occurrence of hydroureter in the absence of any effects on fetal mortality, litter size, fetal body weight, or pregnancy rate. Krejci et al. (1980) showed that tetracycline given by intramuscular injection (total dose: 37.5-70.5 mg on days 6-16 antepartum) into pregnant rats was associated with an increased incidence of discolored corneas and yellow lens opacity in the newborn. Rados (1974) injected oxytetracycline into chicken eggs, which resulted in cessation of embryonic development in 53% of the eggs dosed with 0.03 mg oxytetracycline; 29% of the embryos at this level showed scarcely differentiated mesoderm and entoderm. Bergman (1970) observed that the offspring of tropical fish exposed to tetracycline at 50 mg per gallon of water showed a high incidence of stillbirths, neonatal deaths, deformities of the dorsal spine and tail, and impairment of reproductive capability.

Genetic Toxicology

Tetracycline hydrochloride is positive in the DNA-cell-binding assay, which suggests mutagenic and carcinogenic potential (Kubinski et al., 1981). However, limited information on the genotoxicity of tetracycline hydrochloride indicates that the compound is not mutagenic. Tetracycline hydrochloride was not mutagenic when tested by the NTP in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with a preincubation protocol in the presence or absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Zeiger et al., 1987; Table E1). Mutagenicity testing of antimicrobials in bacterial assay systems can be conducted because the endpoint, gene mutation, may be achieved at concentrations below those that produce toxicity. Tetracycline hydrochloride was also negative in an NTP mouse lymphoma L5178Y/TK$^{+/-}$ assay performed with or without Aroclor 1254-induced male F344 rat liver S9; a marginally positive response in this assay was observed when the chemical was tested in the presence of noninduced S9 (Table E2). In NTP cytogenetic assays with Chinese hamster ovary cells, treatment with tetracycline hydrochloride in the presence or absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 did not induce chromosomal aberrations or sister chromatid exchanges (Tables E3 and E4). In the only reported in vivo study, tetracycline hydrochloride did not induce sex-linked recessive lethal mutations when administered by feeding or injection to adult male Drosophila (Table E5).

There are only two other reports on the mutagenicity of tetracycline hydrochloride; in both studies, cultured mammalian cells were used, and positive responses were obtained. Tsutsui et al. (1976) reported that incubation of cultured FM3A cells from a C3H mouse mammary carcinoma with 10-100 µg/ml tetracycline hydrochloride for 48 hours induced a dose-related increase in the frequency of 8-azaguanine-resistant mutants. They also reported a significant increase in chromosomal aberrations in FM3A cells cultured for 24 and 48 hours with tetracycline hydrochloride. Bhattacharjee and Pal (1982) reported a dose-dependent increase in 8-azaguanine-resistant mutants in Chinese hamster V79 cells after treatment with 10-80 µg/ml tetracycline hydrochloride for 48 hours followed by a 7-day expression time. Bhattacharjee and Pal further observed that treatment of V79 cells with 10 or 20 µg/ml ascorbic acid, either before or after but not concurrently with the 48-hour exposure to tetracycline hydrochloride, markedly reduced the effectiveness of mutation induction.

Study Rationale

Tetracycline hydrochloride was nominated for long-term toxicity and carcinogenicity studies by the National Cancer Institute because of extensive human exposure through its use as an antibiotic and because the available studies were not considered adequate (NCI, 1977). Because of the stability of this compound and because human exposure is usually oral, tetracycline hydrochloride was administered in feed to both rats and mice.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF TETRACYCLINE HYDROCHLORIDE

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

- Study Design
- Source and Specifications of Animals
- Animal Maintenance
- Clinical Examinations and Pathology
- Statistical Methods

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF TETRACYCLINE HYDROCHLORIDE

USP-grade tetracycline hydrochloride was obtained in two lots from Lederle Laboratories (Pearl River, New York) (Table 1). Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, Missouri). MRI reports on analyses performed in support of the tetracycline hydrochloride studies are on file at the National Institute of Environmental Health Sciences. Both lots were obtained as a yellow, microcrystalline solid. Chemical identity was confirmed by spectroscopy. The infrared and nuclear magnetic resonance spectra (Figures 1 to 4) were consistent with spectra in the literature (Sadtler Standard Spectra; Schach von Wittenau and Blackwood, 1966). The ultraviolet/visible spectrum was consistent with that expected for the structure of tetracycline hydrochloride.

Lot no. 48355-725 had a melting point of 221°-225° C with decomposition, as indicated by a darkening and evolution of gas. The purity of lot no. 48355-725 was approximately 91% as determined by elemental analysis, titration of the amine group, thin-layer chromatography, and high-performance liquid chromatography. Results of elemental analyses for hydrogen and nitrogen agreed with the theoretical values; that for carbon was low. The values for both ionic chlorine and total chlorine were high. Water content by Karl Fischer titration was 0.58%. Perchloric acid titration of the amine functional group gave 111.4% and 97.8%, respectively, for the sample and the USP standard. The anomalous titration value and high chlorine content might be explained by the presence of approximately 1.2% ammonium chloride. The relative ultraviolet absorptivity was 98%-99% that of a USP standard at four different absorption maxima. No impurities were detected by thin-layer chromatography on silanized silica gel plates with a water-saturated *n*-butanol solvent system or on cellulose F plates with a 100 mM sodium EDTA:isopropanol (1:1) solvent system; visualization was by ultraviolet light at 254 nm, ammonium hydroxide, and a boric acid/sulfuric acid spray reagent. High-performance liquid chromatography on a μBondapak C_{18} column with a mobile phase of aqueous 1.5 mM quaternary ammonium EDTA containing 5% (v/v) acetic acid: tetrahydrofuran (programmed at 100:0 to 80:20 over 50 minutes) at a flow rate of 1 ml/minute and ultraviolet detection at 254 nm detected four impurities. One impurity had a peak area of 6% that of the major peak, and three impurities had a relative total area of 1.0%.

TABLE 1. IDENTITY AND SOURCE OF TETRACYCLINE HYDROCHLORIDE USED IN THE FEED STUDIES

Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
Lot Numbers		
48355-725	48355-725	48355-725; 48355-1001
Date of Initial Use		
7/1/79	4/14/80	48355-725--2/9/81; 48355-1001--4/5/82
Supplier		
Lederle Laboratories (Pearl River, NY)	Same as 14-d studies	Same as 14-d studies

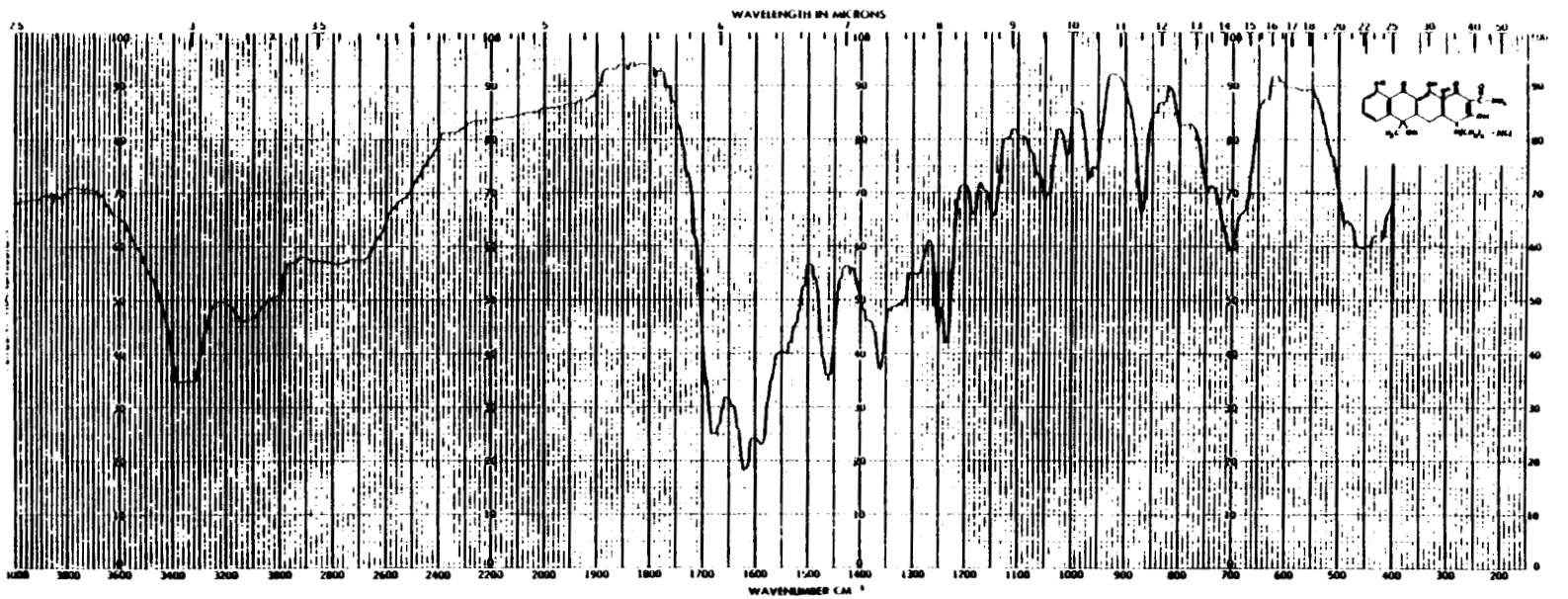

FIGURE 1. INFRARED ABSORPTION SPECTRUM OF TETRACYCLINE HYDROCHLORIDE (LOT NO. 48355-725)

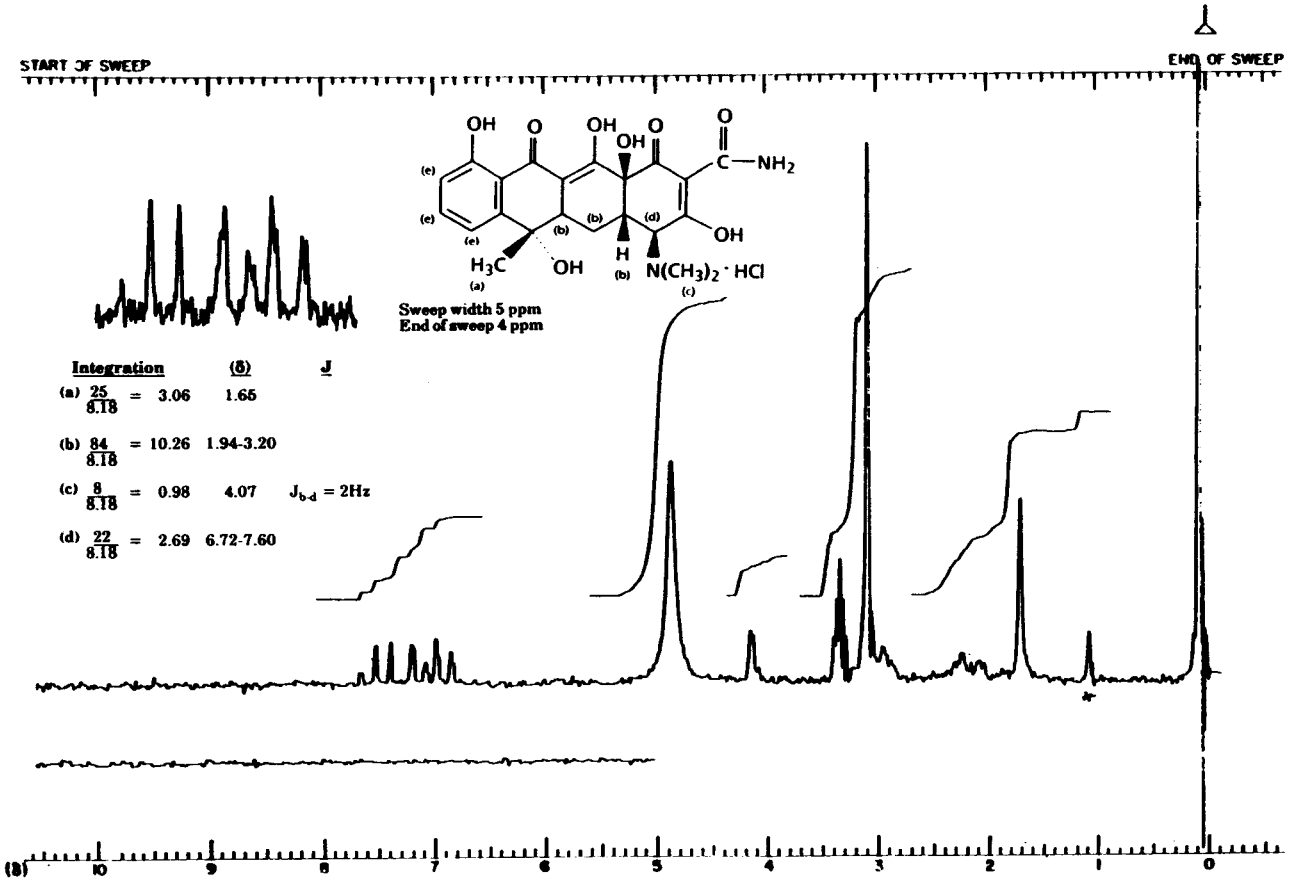

FIGURE 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF TETRACYCLINE HYDROCHLORIDE (LOT NO. 48355-725)

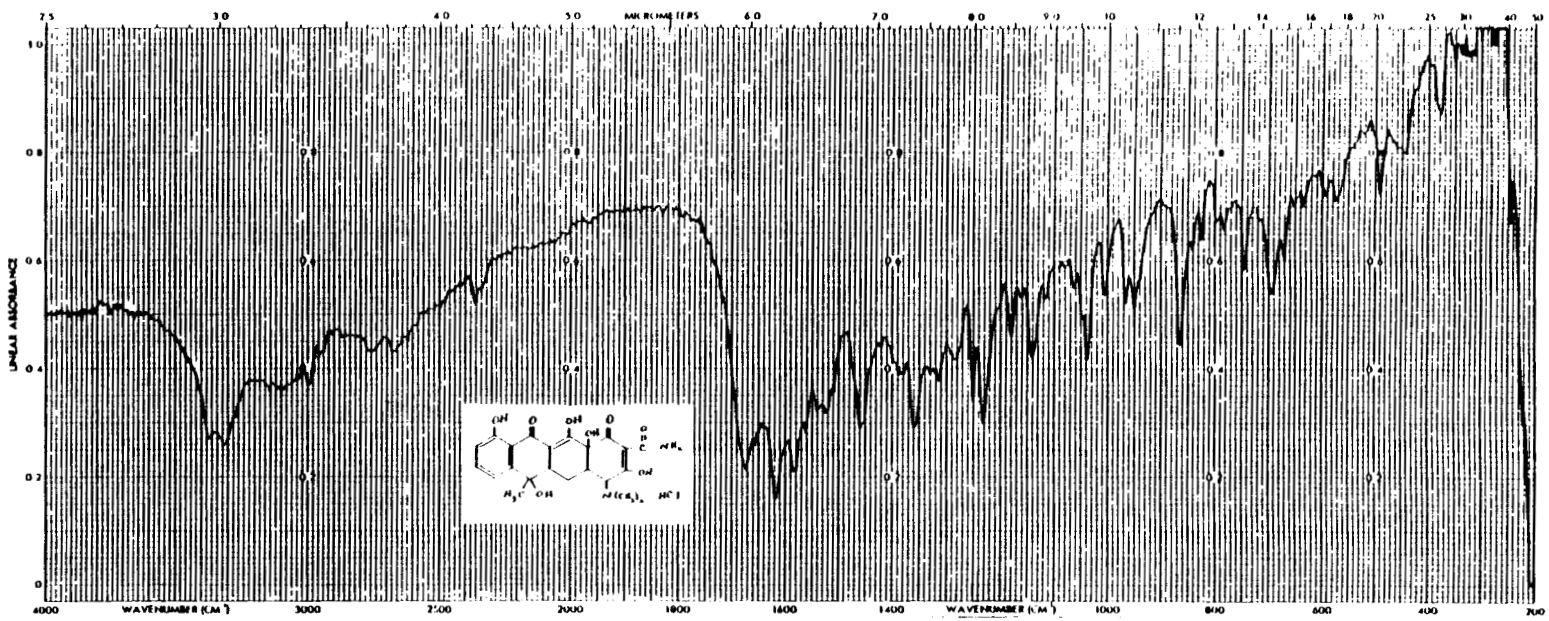

FIGURE 3. INFRARED ABSORPTION SPECTRUM OF TETRACYCLINE HYDROCHLORIDE (LOT NO. 48355-1001)

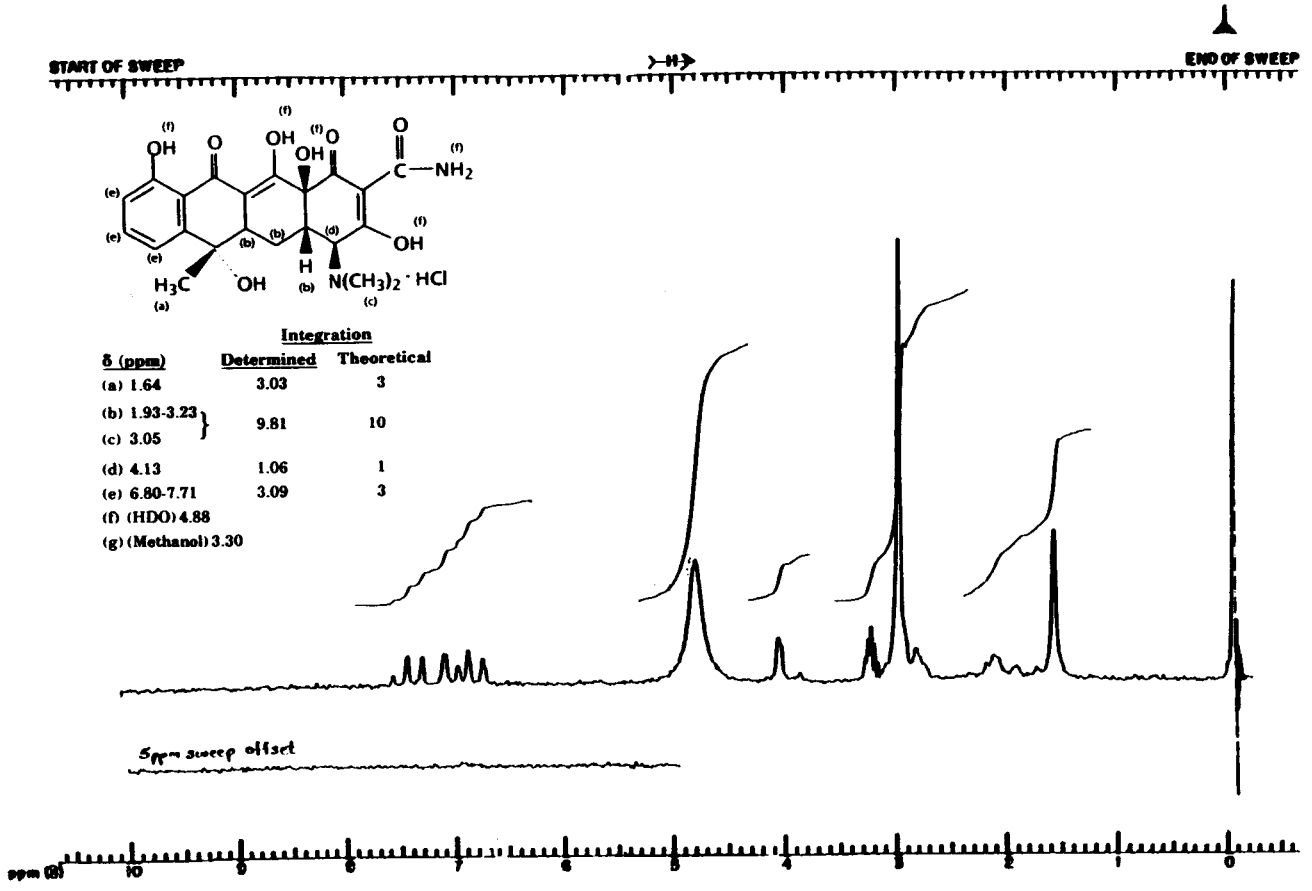

FIGURE 4. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF TETRACYCLINE HYDROCHLORIDE (LOT NO. 48355-1001)

Cumulative data indicated that lot no. 48355-1001 was approximately 92% pure. Results of elemental analyses agreed with the theoretical values for hydrogen and nitrogen; that for carbon was low. The values for total and ionic chlorine were high. Water content was 0.24% by Karl Fischer titration. Perchloric acid titration gave a purity value of 113.3%. Thin-layer chromatography by the same systems that were used for lot no. 48355-725, including visible light detection, indicated no impurities. High-performance liquid chromatography on a μBondapak C_{18} column with a mobile phase of aqueous 1.5 mM quaternary ammonium EDTA containing 5% (v/v) acetic acid:tetrahydrofuran (programmed at 100:0 to 80:20 over 50 minutes) at a flow rate of 1 ml/minute and ultraviolet detection at 254 nm indicated four impurities. One impurity had a peak area of 5% that of the major peak, and three impurities had a relative total area of 1.3%. Comparisons of major peaks by the high-performance liquid chromatographic system described above for lot no. 48355-725, lot no. 48355-1001, and a USP reference standard gave a relative purity of 100.0%, 100.6%, and 100.6%, respectively, normalized to lot no. 48355-725. The USP reference standard also contained the approximately 5% impurity observed in both lots of study material.

Stability studies performed by high-performance liquid chromatography on a μBondapak C_{18} column with a mobile phase of aqueous 2 mM quaternary ammonium EDTA containing 5% (v/v) acetic acid:tetrahydrofuran (75:25) at a flow rate of 1 ml/minute and ultraviolet detection at 254 nm indicated that tetracycline hydrochloride was stable in storage for 2 weeks at 60° C. Further confirmation of the stability of the bulk chemical during the toxicity studies (storage at 25° C) was obtained by titration with 0.1 N perchloric acid and ultraviolet spectroscopy versus a reference standard. No degradation was seen over the course of the studies. Identity of the chemical was confirmed by infrared spectroscopy.

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

The formulated diets were prepared by adding a dry premix to the appropriate amount of feed and blending for 15 minutes (Table 2). Homogeneity of diet mixtures formulated at the analytical chemistry and study laboratories was evaluated by extracting feed samples (taken from three locations within the blender) with acidic methanol (1 or 5 ml concentrated hydrochloric

TABLE 2. PREPARATION AND STORAGE OF FORMULATED DIETS IN THE FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
Preparation For each dietary concentration, premix prepared by mixing weighed chemical with weighed feed in a beaker, adding additional feed, and mixing with a spatula; premix layered with remaining feed in an 8-qt Patterson-Kelly Twin-Shell® blender and blended with intensifier bar on for 5 min and off for 10 min	Same as 14-d studies	Same as 14-d studies, except that blending done in a 1-ft^3 Patterson-Kelly Twin-Shell® blender
Maximum Storage Time 1 wk	2 wk	2 wk
Storage Conditions In light-restricted containers at room temperature	Same as 14-d studies	Same as 14-d studies

acid per liter of methanol) and analyzing the extracts by ultraviolet spectroscopy at 269 nm (analytical chemistry laboratory) or high-performance liquid chromatography on a μBondapak C_{18} column with a mobile phase of aqueous 1.5 mM quaternary ammonium EDTA containing 5% (v/v) acetic acid:tetrahydrofuran (86:14) at a flow rate of 2 ml/minute and with detection at 254 nm (study laboratory). Good homogeneity was found at the analytical chemistry laboratory; concentrations of samples taken from three locations within the blender ranged from 100% to 101% that of the target concentration of 10,000 ppm. At the study laboratory, values ranged from 94.7% to 97.0% that of the target concentration of 50,000 ppm and from 93.5% to 100.3% at 3,100 ppm. Further studies conducted at the analytical chemistry laboratory with the same high-performance liquid chromatographic system, except with a mobile phase of aqueous 1.5 mM quaternary ammonium EDTA containing 5% (v/v) acetic acid:tetrahydrofuran (88:12), showed that tetracycline hydrochloride at 10,000 ppm was stable in feed when stored in the dark for at least 2 weeks at 25° C (to simulate actual storage conditions); a 9% decrease was observed at 45° C. One-week stability was confirmed for diets containing 10,000 ppm tetracycline hydrochloride when stored at 25° C under normal animal-room lighting conditions. Each tetracycline hydrochloride mix was used for no longer than 1 week.

Analysis of formulated diets for tetracycline hydrochloride was conducted by the study and analytical chemistry laboratories to determine if the diets contained the correct concentrations of tetracycline hydrochloride. Samples were extracted with acidic methanol and analyzed by ultraviolet spectroscopy (2-year studies) or with the high-performance liquid chromatographic system described above (13-week studies). Formulated diets were analyzed once before the start of the 13-week studies; concentrations of tetracycline hydrochloride ranged from 93% to 99% that of the target concentration (Table 3).

During the 2-year studies, the formulated diets were analyzed periodically, with concentrations varying from 93% to 106% that of the target concentration (Table 4). Because 28/28 feed mixtures analyzed were within 10% of the target concentrations, it is estimated that the feed mixtures were prepared within specifications 100% of the time. Referee analysis was performed periodically by the analytical chemistry laboratory. Good agreement was generally found between the results of the analytical chemistry and study laboratories (Table 5).

TABLE 3. RESULTS OF ANALYSIS OF FORMULATED DIETS BEFORE THE THIRTEEN-WEEK FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE (a)

Concentration of Tetracycline Hydrochloride in Feed (ppm) (b)		Determined as a Percent of Target
Target	Determined	
3,100	3,060	98.7
6,300	6,250	99.2
12,500	12,010	96.1
25,000	23,290	93.2
50,000	47,870	95.7

(a) Mix date: 3/18/80
(b) Results of duplicate analysis

TABLE 4. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

Date Mixed	Concentration of Tetracycline Hydrochloride in Feed for Target Concentration (ppm) (a)	
	12,500	25,000
02/02/81	13,200	25,200
03/23/81	13,100	26,100
04/20/81	12,100	24,200
06/01/81	11,600	23,800
08/31/81	12,000	24,300
09/21/81	12,300	26,300
11/09/81	12,600	24,500
02/22/82	11,800	24,800
03/01/82	12,500	24,500
05/10/82	12,100	24,200
07/26/82	12,700	23,300
09/06/82	12,300	24,700
10/11/82	12,600	25,100
12/27/82	12,300	24,200
Mean (ppm)	12,371	24,657
Standard deviation	453	817
Coefficient of variation (percent)	3.7	3.3
Range (ppm)	11,600-13,200	23,300-26,300
Number of samples	14	14

(a) Results of duplicate analysis

TABLE 5. RESULTS OF REFEREE ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory (a)	Referee Laboratory (b)
02/02/81	12,500	13,200	12,000
08/31/81	25,000	24,300	24,200
03/01/82	12,500	12,500	12,050
09/06/82	25,000	24,700	24,700

(a) Results of duplicate analysis
(b) Results of triplicate analysis

II. MATERIALS AND METHODS

FOURTEEN-DAY STUDIES

Four- to five-week-old male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories and held for 17 days before the studies began. Groups of five rats and five mice of each sex were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm tetracycline hydrochloride for 14 consecutive days. Rats and mice were observed twice per day and weighed once per week. A necropsy was performed on all animals. Further experimental details are given in Table 6.

THIRTEEN-WEEK STUDIES

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

Five- to six-week-old male and female F344/N rats and 4- to 5-week-old male and female B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories and observed for 14 days. All animals whose weights fell within ±10%-15% of an estimated mean weight were then randomly assigned numbers and cages.

Groups of 10 or 15 rats and 10 or 15 mice of each sex were given diets containing 0, 3,100, 6,300, 12,500, 25,000, or 50,000 ppm tetracycline hydrochloride for 13 weeks. Control diets consisted of NIH 07 Rat and Mouse Ration. Formulated or control diets and water were available ad libitum. Further experimental details are summarized in Table 6.

Animals were observed twice per day; moribund animals were killed. Feed consumption was measured once per week by cage. Individual animal weights were recorded once per week. The concentration of tetracycline in bone was determined by extracting the left femurs of five rats or mice of each sex from the control and 3,100-, 12,500-, and 50,000-ppm groups for 2.5 hours with 0.5 N hydrochloric acid and determining the absorbance at 296 nm.

At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or missing. Tissues examined are listed in Table 6.

TWO-YEAR STUDIES

Study Design

Diets containing 0, 12,500, or 25,000 ppm tetracycline hydrochloride were fed to groups of 50 rats and 50 mice of each sex for 103 weeks.

Source and Specifications of Animals

The male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female × C3H/HeN MTV$^-$, male) mice used in these studies were produced under strict barrier conditions at Charles River Breeding Laboratories under a contract to the Carcinogenesis Program. 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. Animals were quarantined at the study facility for 13 days (rats) or 12 days (mice). Thereafter, a complete necropsy was performed on five 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 7-8 weeks of age. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix F).

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to produce the hybrid B6C3F$_1$ study animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoresis profiles that demonstrate phenotype expressions of known genetic loci.

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

Fourteen-Day Studies	Thirteen-Week Studies	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 except 15 male rats and 15 male mice for the 0, 3,100-, 12,500-, and 50,000-ppm groups	50 males and 50 females of each species
Doses		
0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm tetracycline hydrochloride in feed	0, 3,100, 6,300, 12,500, 25,000, or 50,000 ppm in feed	0, 12,500, or 25,000 ppm tetracycline hydrochloride in feed
Date of First Dose		
7/1/79	4/14/80	Rats--2/9/81; mice--2/23/81
Date of Last Dose		
7/14/79	7/13/80	Rats--1/30/83; mice--2/13/83
Duration of Dosing		
14 consecutive d	13 wk	103 wk
Type and Frequency of Observation		
Observed 2 × d; weighed initially and 1 × wk thereafter	Same as 14-d studies	Observed 2 × d; weighed initially, 1 × wk for 13 wk, and 1 × mo thereafter
Necropsy and Histologic Examinations		
Necropsy performed on all animals; 1 animal of each sex for 12,500-, 25,000-, or 50,000-ppm groups was examined histologically; tissues examined include: adrenal glands, bone marrow, brain, colon, duodenum, esophagus, eyes, gallbladder (mice), gross lesions, heart, kidneys, liver, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes/seminal vesicles or ovaries/uterus, regional lymph nodes, rib from costochrondral junction, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder	Necropsy performed on all animals; the following tissues examined histologically for control and high dose groups, all lower dose animals with lesions, and all rats dying before the end of the studies: adrenal glands, bone marrow, brain, colon, duodenum, esophagus, gross lesions and tissue masses, heart, kidneys, liver, lungs and bronchi, mammary gland, mandibular lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes/seminal vesicles or ovaries/uterus, salivary glands, skin, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Bone fluorescence and ultraviolet absorption studies performed on the male control and 3,100-, 12,500-, and 50,000-ppm groups	Necropsy performed on all animals; the following tissues examined histologically for control and high dose groups: adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), femur or sternebrae or vertebrae including marrow, gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, small intestine, spinal cord (if neurologic signs present), spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. The following tissues were examined in low dose animals: adrenal glands, kidneys, liver, lungs, pancreas, preputial gland, prostate, and spleen in male rats; adrenal glands, liver, and thyroid gland in female rats; mesenteric lymph nodes, spleen, and thymus in male mice; liver, lungs, pituitary gland, spleen, and thyroid gland in female mice
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species		
F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice
Animal Source		
Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE (Continued)

Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Study Laboratory Physiological Research Laboratories	Physiological Research Laboratories	Physiological Research Laboratories
Method of Animal Identification Rats--tail mark; mice--ear punch	Toe clip	Toe clip and ear notch
Time Held Before Study 17 d	14 d	Rats--13 d; mice--12 d
Age When Placed on Study 6-7 wk	Rats--7-8 wk; mice--6-7 wk	Rats--6-7 wk; mice--7-8 wk
Age When Killed 8-9 wk	Rats--20-21 wk; mice--19-20 wk	Rats--110-111 wk; mice--111-112 wk
Necropsy Dates Rats--7/17/79; mice--7/16/79	Rats--7/14/80-7/16/80; mice--7/15/80-7/17/80	Rats--2/7/83-2/14/83; mice--2/21/83-2/28/83
Method of Animal Distribution Animals within ±10%-15% of estimated mean weight were randomly assigned numbers and cages	Same as 14-d studies	Same as 14-d studies
Feed Rodent Laboratory Chow 5001® meal (Ralston Purina Co., St. Louis, MO); available ad libitum	NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	Same as 13-wk studies
Bedding Heat-treated aspen wood shavings (Minnesota Sawdust and Shavings Co., Anoka, MN)	Same as 14-d studies	Same as 14-d studies
Water Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as 14-d studies	Same as 14-d studies
Cages Polycarbonate (Lab Products, Garfield, NJ)	Polycarbonate (Hazleton Systems, Aberdeen, MD)	Same as 13-wk studies
Cage Filters Reemay spun-bonded polyester filters (Snow Filtration, Cincinnati, OH)	Same as 14-d studies	Same as 14-d studies
Animals per Cage 5	5	5
Other Chemicals on Study in the Same Room None	None	None
Animal Room Environment Temp--21.1°-25.0° C; hum--40%-70%; fluorescent light 12 h/d	Temp--18.8°-25.5° C; hum--40%-56%; fluorescent light 12 h/d	Temp--21.7°-26.7° C; hum--30%-70%; fluorescent light 12 h/d (two light intensities were used)

The C57BL/6N mice were homogeneous at all loci tested. Eighty-five percent of the C3H mice monitored were variant at one to three loci, indicating some heterogeneity in the C3H line from this supplier. Nevertheless, the genome of this line is more homogeneous than that of randomly bred stocks.

Male mice from the C3H colony and female mice from the C57BL/6N colony were used as parents for the hybrid B6C3F$_1$ mice used in these studies. The influence of the potential genetic nonuniformity in the hybrid mice on these results is not known, but results of the studies are not affected because concurrent controls were included in each study.

Animal Maintenance

Animals were housed five per cage. Feed and water were available ad libitum. Further details of animal maintenance are given in Table 6.

Clinical Examinations and Pathology

All animals were observed two times per day, and clinical signs were recorded at least once per month. Body weights were recorded once per week for the first 13 weeks of the study and 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, unless they were excessively autolyzed or cannibalized or missing. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study.

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 examination of tissues was performed according to an "inverse pyramid" design (McConnell, 1983a,b). That is, complete histopathologic examinations (Table 6) were performed on all high dose and control animals and on low dose animals dying before the end of the study. In addition, histopathologic examinations were performed on all grossly visible lesions in all dose groups. Potential target organs for chemically related neoplastic and nonneoplastic effects were identified from the short-term studies or the literature and were determined by examination of the pathology data; these target organs/tissues in the lower dose group were examined histopathologically. If mortality in the highest dose group exceeded that in the control group by 15%, complete histopathologic examinations were performed on all animals in the second highest dose group in addition to those in the high dose group.

When the pathology evaluation was completed, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assessment pathologist. The quality assessment report and slides were submitted to the Pathology Working Group (PWG) Chairperson, who reviewed all target tissues and those about which there was a disagreement between the laboratory and quality assessment pathologists.

Representative slides selected by the Chairperson were reviewed by the PWG, which included the laboratory pathologist, without knowledge of previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the laboratory pathologist was asked to reconsider the original diagnosis. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses 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).

Slides/tissues are generally not evaluated in a blind fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle or unless there is an inconsistent diagnosis of lesions by the laboratory pathologist. Nonneoplastic lesions are not examined routinely by the quality assessment pathologist or PWG unless they are considered part of the toxic effect of the chemical.

Statistical Methods

Data Recording: Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

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 at the time they were found dead of other than natural causes or were found to be missing; 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: Three statistical methods are used to analyze tumor incidence data: life table tests, incidental tumor analysis, and Fisher exact/Cochran-Armitage trend analyses. Tests of significance include pairwise comparisons of high dose and low dose groups with controls and tests for overall dose-response trends. For studies in which administration of the test compound has little effect on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. Continuity-corrected tests are used in the analysis of tumor incidence, and reported P values are one-sided. The procedures described below also were used to evaluate selected nonneoplastic lesions.

Life Table Analysis--The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel method (1959) to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975). The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time to death due to tumor closely approximates time to tumor onset. In this case, the life table test also provides a comparison of the time-specific tumor incidences.

Incidental Tumor Analysis--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the study were "incidental"; i.e., they were merely observed at necropsy in animals

dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals actually examined for tumors during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.)

Fisher Exact/Cochran-Armitage Trend Analyses--In addition to survival-adjusted methods, the results of the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) are given in the appendixes containing the analyses of tumor incidence. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

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, Feed Consumption, and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

MICE

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

FOURTEEN-DAY STUDIES

None of the rats died before the end of the studies (Table 7). The final mean body weight of male rats that received 25,000 or 50,000 ppm was 7% or 24% lower than that of the controls. The final mean body weights of dosed female rats were similar to that of the controls, although the amount of weight gained was less in the high dose than in the control group. Feed consumption was lower in the high dose groups than in the controls during the first week of the studies but was comparable during the second. Lethargy and rough coats were observed for rats that received 25,000 or 50,000 ppm. No compound-related effects were observed at necropsy.

THIRTEEN-WEEK STUDIES

None of the rats died before the end of the studies (Table 8). The final mean body weight of male rats that received 50,000 ppm was 18% lower than that of the controls. Final mean body weights of dosed and control female rats were similar. Feed consumption by the dosed groups was generally similar to that by the controls after the first week of the studies.

Cytoplasmic vacuolization was diagnosed in the liver of 10/10 males at 50,000 ppm and 9/10 males at 25,000 ppm. Mild bone marrow atrophy was observed in 3/10 males at 50,000; minimal bone marrow atrophy was observed in 2/10 males at 50,000 ppm, 3/10 males at 25,000 ppm, and 1/10 males at 12,500 ppm.

Minimal or mild bone marrow atrophy was found in 3/10 females at 50,000 ppm, 5/10 females at 25,000 ppm, and 7/10 females at 12,500 ppm. The concentration of tetracycline in bone increased with increasing dose of tetracycline hydrochloride (Table 9).

Dose Selection Rationale: Because of the effect on mean body weights in males and histopathologic effects in the liver and bone marrow, dietary concentrations of 0, 12,500, and 25,000 ppm tetracycline hydrochloride were selected for rats for the 2-year studies.

TABLE 7. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE FOURTEEN-DAY FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 1	Week 2
MALE							
0	5/5	117 ± 5	197 ± 5	+80 ± 3		13.8	15.5
3,125	5/5	117 ± 1	205 ± 1	+88 ± 1	104	14.8	16.5
6,250	5/5	118 ± 2	200 ± 3	+82 ± 4	102	13.7	15.6
12,500	5/5	113 ± 5	194 ± 6	+81 ± 4	98	14.3	16.7
25,000	5/5	111 ± 3	184 ± 1	+73 ± 4	93	11.3	15.8
50,000	5/5	116 ± 4	149 ± 5	+33 ± 2	76	10.4	13.9
FEMALE							
0	5/5	92 ± 2	126 ± 2	+34 ± 1		9.6	10.1
3,125	5/5	93 ± 1	131 ± 1	+38 ± 1	104	8.8	12.1
6,250	5/5	100 ± 1	138 ± 1	+38 ± 1	110	10.2	11.4
12,500	5/5	94 ± 1	133 ± 1	+39 ± 1	106	9.7	11.0
25,000	5/5	95 ± 2	133 ± 4	+38 ± 2	106	8.7	11.9
50,000	5/5	99 ± 2	126 ± 3	+27 ± 1	100	6.3	11.7

(a) Number surviving/number initially in the group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.

TABLE 8. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 6	Week 13
MALE							
0	15/15	122 ± 2	333 ± 4	+211 ± 3		14.4	14.9
3,100	15/15	133 ± 2	328 ± 3	+195 ± 3	98	14.9	13.3
6,300	10/10	127 ± 2	323 ± 8	+196 ± 6	97	14.9	13.8
12,500	15/15	129 ± 1	326 ± 4	+197 ± 4	98	15.2	14.3
25,000	10/10	123 ± 2	305 ± 6	+182 ± 6	92	14.3	13.7
50,000	15/15	121 ± 2	272 ± 4	+151 ± 3	82	13.5	13.4
FEMALE							
0	10/10	101 ± 1	194 ± 2	+93 ± 2		11.2	10.1
3,100	10/10	105 ± 2	197 ± 3	+92 ± 2	102	10.3	9.4
6,300	10/10	98 ± 1	191 ± 3	+93 ± 4	98	10.7	9.3
12,500	10/10	102 ± 2	193 ± 2	+91 ± 2	99	10.6	10.4
25,000	10/10	100 ± 2	189 ± 2	+89 ± 2	97	11.2	10.5
50,000	10/10	97 ± 2	188 ± 3	+91 ± 3	97	10.6	10.9

(a) Number surviving/number initially in the group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.

TABLE 9. CONCENTRATION OF TETRACYCLINE IN BONE OF RATS IN THE THIRTEEN-WEEK FEED STUDIES (a)

	Tetracycline Hydrochloride in Feed (ppm) 3,100	12,500	50,000
MALE	27	119	457
FEMALE	21	168	580

(a) Mean micrograms/gram of bone for left femurs of five animals

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Mean body weights of dosed male and female rats were similar to those of controls throughout the studies (Table 10 and Figure 5). The average daily feed consumption per rat by dosed and control male and female rats was also similar (Tables G1 and G2). The average amount of tetracycline hydrochloride consumed per day was approximately 440 or 910 mg/kg for low dose or high dose male rats and 510 or 1,060 mg/kg for low dose or high dose female rats. No compound-related clinical signs were observed.

TABLE 10. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

Weeks on Study	Control		12,500 ppm			25,000 ppm		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE								
1	152	50	146	96	50	143	94	50
2	194	50	187	96	50	184	95	50
3	226	50	216	96	50	215	95	50
4	255	50	245	96	50	239	94	50
5	279	50	269	96	50	260	93	50
6	299	50	287	96	50	279	93	50
7	309	50	296	96	50	290	94	50
8	327	50	310	95	50	306	94	50
9	338	50	320	95	50	318	94	50
10	351	50	335	95	50	331	94	50
11	358	50	344	96	50	342	96	50
12	362	50	354	98	50	355	98	50
13	377	50	366	97	50	364	97	50
19	402	50	399	99	50	393	98	50
24	430	50	420	98	50	415	97	50
29	452	50	437	97	50	433	96	50
32	452	50	441	98	50	432	96	50
37	461	50	450	98	50	444	96	50
41	468	50	459	98	50	453	97	49
45	478	50	467	98	50	461	96	49
50	478	50	471	99	50	466	97	49
54	476	49	471	99	50	465	98	49
58	469	48	468	100	50	457	97	49
63	474	48	471	99	50	462	97	49
67	484	47	474	98	50	465	96	48
71	462	47	460	100	50	448	97	48
76	452	47	463	102	49	439	97	48
80	450	45	453	101	47	439	98	46
84	444	44	441	99	47	436	98	45
88	433	42	437	101	44	425	98	45
93	449	38	438	98	39	431	96	42
97	438	36	440	100	32	425	97	39
101	424	32	419	99	29	418	99	38
104	421	27	410	97	24	413	98	31
FEMALE								
1	120	50	120	100	50	113	94	50
2	142	50	142	100	50	142	100	50
3	154	50	156	101	50	153	99	50
4	164	50	166	101	50	162	99	50
5	175	50	177	101	50	169	97	50
6	186	50	182	98	50	178	96	50
7	188	50	188	100	50	183	97	50
8	194	50	195	101	50	185	95	50
9	196	50	198	101	50	190	97	50
10	201	50	204	101	50	196	98	50
11	204	50	207	101	50	202	99	50
12	208	50	210	101	50	207	100	50
13	211	50	214	101	50	210	100	50
19	219	50	224	102	50	217	99	50
24	228	50	235	103	50	225	99	49
29	234	50	241	103	50	234	100	49
32	240	50	244	102	50	235	98	49
37	245	50	252	103	50	244	100	49
41	250	50	260	104	49	248	99	49
45	258	50	266	103	49	254	98	49
50	268	50	273	102	49	259	97	49
54	270	48	279	103	49	263	97	49
58	277	47	287	104	49	269	97	49
63	285	47	294	103	49	273	96	48
67	295	47	305	103	49	282	96	48
71	299	47	310	104	49	287	96	48
76	306	45	317	104	49	298	97	48
80	298	45	307	103	48	290	97	48
84	294	43	312	106	48	295	100	47
88	300	40	319	106	45	294	98	45
93	315	36	328	104	44	302	96	43
97	318	31	330	104	43	306	96	41
101	317	28	319	101	41	304	96	39
104	324	27	331	102	39	322	99	38

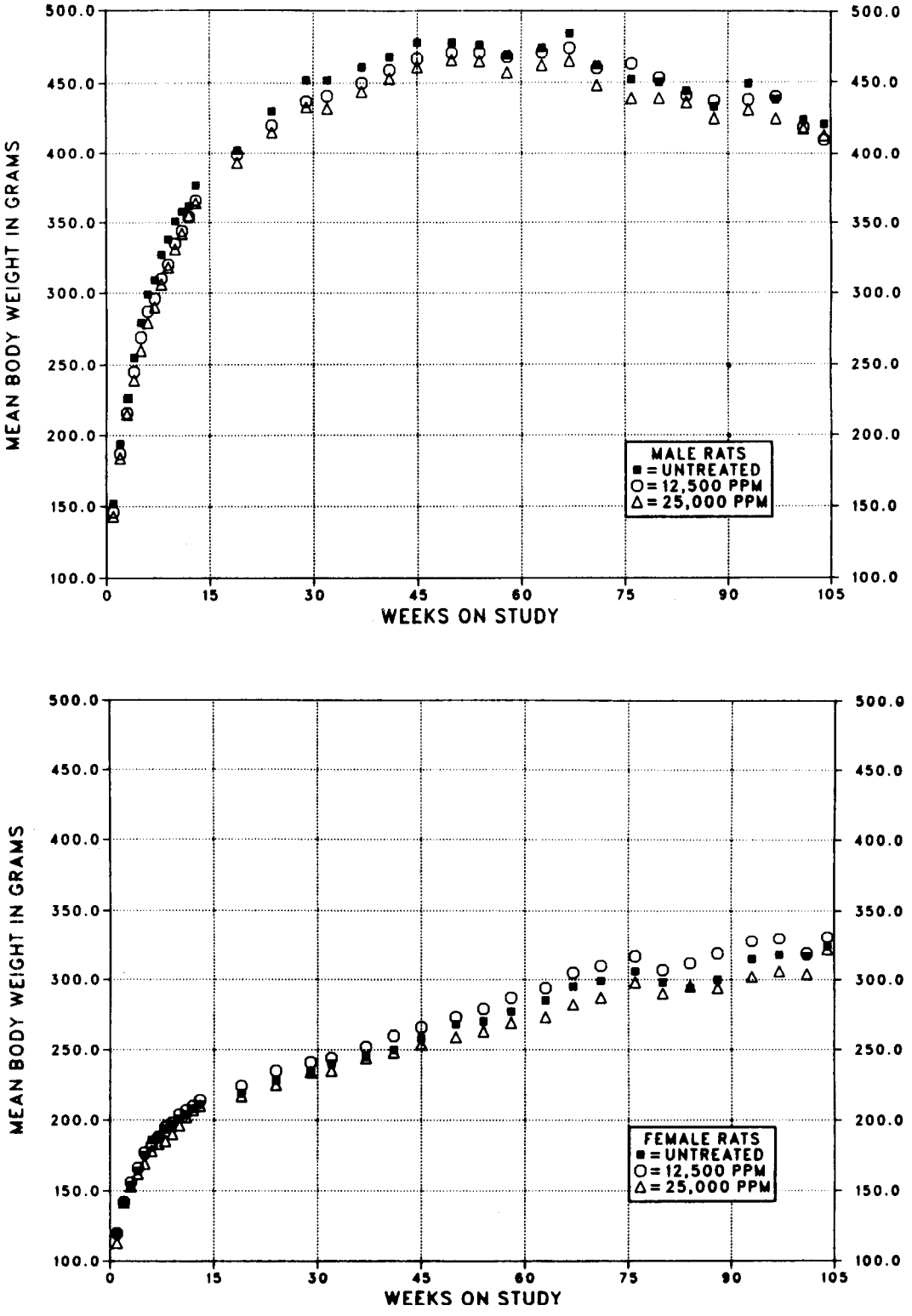

FIGURE 5. GROWTH CURVES FOR RATS FED DIETS CONTAINING TETRACYCLINE HYDROCHLORIDE FOR TWO YEARS

III. RESULTS: RATS

Survival

Estimates of the probabilities of survival for male and female rats fed diets containing tetracycline hydrochloride at the concentrations used in these studies and for controls are shown in Table 11 and in the Kaplan and Meier curves in Figure 6. The survival of both the low (after week 95) and high (at the end of the study) dose female groups was significantly greater than that of the controls. No significant differences in survival were observed between any groups of male rats.

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 pancreatic islets, liver, anterior pituitary gland, hematopoietic system, and kidney.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors 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 11. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

	Control	12,500 ppm	25,000 ppm
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	23	26	19
Animals surviving until study termination	27	24	31
Survival P values (c)	0.409	0.751	0.455
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	22	11	12
Animals missing	1	0	0
Animals surviving until study termination	27	39	38
Survival P values (c)	0.027	0.021	0.044

(a) Termination period: week 104
(b) Includes animals killed in a moribund condition
(c) 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.

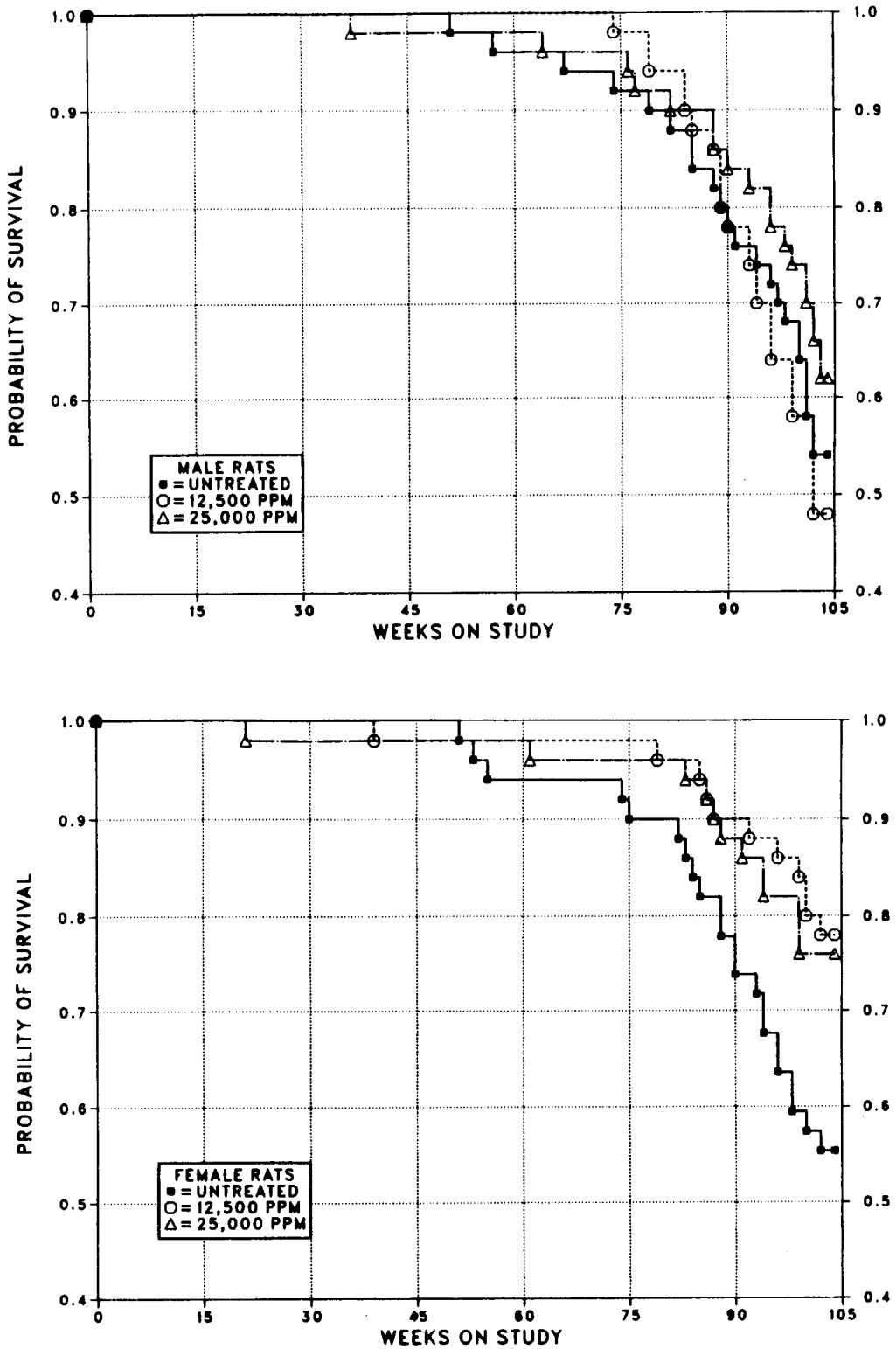

FIGURE 6. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING TETRACYCLINE HYDROCHLORIDE FOR TWO YEARS

Pancreatic Islets: The incidence of adenomas or carcinomas (combined) in low dose male rats was significantly greater than that in the controls (Table 12).

Liver: Basophilic cytoplasmic change and clear cell change were observed at increased incidences in dosed male rats (basophilic cytoplasmic change--male: control, 12/50; low dose, 20/49; high dose, 28/50; $P<0.05$; female: none observed; clear cell change--male: 5/50; 7/49; 13/50; $P=0.052$; female: none observed). The incidences of bile duct hyperplasia were decreased in dosed male and female rats (male: 44/50; 35/49; 26/50; $P<0.01$; female: 34/49; 24/50; 23/50; $P<0.025$).

Anterior Pituitary Gland: Focal hyperplasia was observed at increased incidences in dosed rats (male: control, 4/48; low dose, 11/49; high dose, 9/50; female: 5/48; 8/43; 15/50; $P=0.013$). Adenomas or carcinomas (combined) in female rats occurred with a significant negative trend (26/48; 24/43; 19/50; $P<0.05$); the incidences in the dosed groups were not significantly different from that in the controls by the incidental tumor test.

TABLE 12. PANCREATIC ISLET CELL LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (a)

	Control	12,500 ppm (b)	25,000 ppm (b)
Hyperplasia			
Overall Rates	0/49 (0%)	1/49 (2%)	0/49 (0%)
Adenoma			
Overall Rates	0/49 (0%)	3/49 (6%)	0/49 (0%)
Carcinoma			
Overall Rates	0/49 (0%)	2/49 (4%)	0/49 (0%)
Adenoma or Carcinoma (c)			
Overall Rates	0/49 (0%)	5/49 (10%)	0/49 (0%)
Adjusted Rates	0.0%	17.8%	0.0%
Terminal Rates	0/27 (0%)	3/23 (13%)	0/31 (0%)
Week of First Observation		79	
Life Table Tests	P=0.562N	P=0.028	(d)
Incidental Tumor Tests	P=0.609	P=0.037	(d)

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table A3 (footnotes).
(b) The estimated dose in milligrams per kilograms per day is given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix G.
(c) Historical incidence at study laboratory (mean ± SD): 9/148 (6% ± 5%); historical incidence in NTP studies: 100/1,871 (5% ± 4%)
(d) No P value is reported because no tumors were observed in the 25,000-ppm and control groups.

Hematopoietic System: Mononuclear cell leukemia in male rats occurred with a significant negative trend; the incidence in the high dose group was significantly lower than that in the controls (Table 13).

Kidney: The incidences and severity of nephropathy were reduced in dosed male rats (control, 48/50 [2.8 severity]; low dose, 35/50 [1.6 severity]; high dose, 36/50 [1.5 severity]; $P<0.001$).

TABLE 13. MONONUCLEAR CELL LEUKEMIA IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (a)

	Control	12,500 ppm	25,000 ppm
Overall Rates	36/50 (72%)	39/50 (78%)	24/50 (48%)
Adjusted Rates	83.4%	86.1%	53.8%
Terminal Rates	20/27 (74%)	18/24 (75%)	11/31 (35%)
Week of First Observation	51	74	76
Life Table Tests	P=0.012N	P=0.254	P=0.013N
Incidental Tumor Tests	P=0.009N	P=0.415	P=0.012N

(a) Historical incidence of leukemia at study laboratory (mean ± SD): 71/150 (47% ± 3%); historical incidence in NTP studies: 586/1,937 (30% ± 12%)

III. RESULTS: MICE

FOURTEEN-DAY STUDIES

None of the mice died before the end of the studies (Table 14). The final mean body weight of mice that received 25,000 or 50,000 ppm in the diet was 6% or 18% lower than that of the controls for males and 12% or 15% lower for females. Feed consumption was reduced approximately 40% for the 50,000-ppm males during weeks 1 and 2 and for females in the 25,000- and 50,000-ppm groups during week 1. Rough hair coats, lethargy, and unthriftiness were observed for males that received 6,250, 12,500, 25,000, or 50,000 ppm and for females that received 12,500 or 25,000 ppm. Trembling was observed for males that received 12,500 or 25,000 ppm and for females that received 12,500, 25,000, or 50,000 ppm. No compound-related effects were observed at necropsy.

THIRTEEN-WEEK STUDIES

None of the mice died before the end of the studies (Table 15). The final mean body weight of male mice that received 25,000 or 50,000 ppm was 5% or 16% lower than that of the controls. The final mean body weight of female mice that received 50,000 ppm was 6% lower than that of the controls. Estimated feed consumption by dosed groups was similar to that by the controls.

The concentration of tetracycline in bone increased with increasing dose (Table 16). No compound-related clinical signs or gross or microscopic pathologic effects were observed.

Dose Selection Rationale: Because body weight gain for male and female mice receiving 50,000 ppm tetracycline hydrochloride in feed (13-week studies) was 68% and 83% of comparable control values, tetracycline hydrochloride doses selected for the 2-year studies were 0, 12,500, and 25,000 ppm in feed.

TABLE 14. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE FOURTEEN-DAY FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 1	Week 2
MALE							
0	5/5	23.1 ± 0.7	23.6 ± 0.9	+0.5 ± 0.3		2.8	3.2
3,125	5/5	21.8 ± 0.2	24.8 ± 0.9	+3.0 ± 0.7	105	3.0	3.2
6,250	5/5	(e) 22.8 ± 1.4	23.4 ± 1.3	+0.5 ± 0.3	99	2.8	3.0
12,500	5/5	24.2 ± 1.1	25.4 ± 1.2	+1.2 ± 0.5	108	2.5	2.9
25,000	5/5	22.0 ± 1.2	22.2 ± 1.5	+0.2 ± 1.1	94	2.3	2.8
50,000	5/5	22.6 ± 0.5	19.4 ± 0.8	−3.2 ± 0.9	82	1.6	2.0
FEMALE							
0	5/5	19.7 ± 0.3	21.4 ± 0.5	+1.7 ± 0.2		3.0	2.8
3,125	5/5	19.7 ± 0.6	19.8 ± 0.5	+0.1 ± 0.2	93	3.0	3.3
6,250	5/5	20.4 ± 0.7	21.6 ± 0.7	+1.2 ± 0.2	101	3.0	3.1
12,500	5/5	19.0 ± 0.7	19.4 ± 0.7	+0.4 ± 0.3	91	2.2	2.7
25,000	5/5	18.5 ± 0.5	18.8 ± 0.4	+0.3 ± 0.4	88	1.9	2.4
50,000	5/5	19.7 ± 0.3	18.2 ± 0.5	−1.5 ± 0.6	85	1.8	2.3

(a) Number surviving/number initially in the group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.
(e) One animal not weighed initially; final body weight is for five animals; initial weight and weight change based on four animals.

TABLE 15. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 6	Week 13
MALE							
0	15/15	21.5 ± 0.4	34.6 ± 0.6	+13.1 ± 0.6		3.6	3.9
3,100	15/15	19.2 ± 0.3	32.1 ± 0.4	+12.9 ± 0.4	93	3.3	3.6
6,300	10/10	17.2 ± 1.1	33.1 ± 0.6	+15.9 ± 1.6	96	3.5	3.8
12,500	15/15	17.8 ± 0.4	30.1 ± 0.5	+12.3 ± 0.5	87	3.2	3.6
25,000	10/10	22.1 ± 0.5	32.9 ± 0.8	+10.8 ± 0.8	95	3.6	3.7
50,000	15/15	20.2 ± 0.2	29.1 ± 0.2	+8.9 ± 0.4	84	3.7	4.4
FEMALE							
0	10/10	16.9 ± 0.5	23.3 ± 0.5	+6.4 ± 0.3		2.7	3.2
3,100	10/10	16.2 ± 0.2	24.8 ± 0.5	+8.6 ± 0.5	106	2.8	3.0
6,300	10/10	19.1 ± 0.2	25.8 ± 0.7	+6.7 ± 0.6	111	2.8	3.0
12,500	10/10	18.4 ± 0.3	25.2 ± 0.5	+6.8 ± 0.5	108	2.8	3.2
25,000	10/10	19.3 ± 0.1	24.9 ± 0.4	+5.6 ± 0.4	107	2.9	3.0
50,000	10/10	16.6 ± 0.2	21.9 ± 0.5	+5.3 ± 0.6	94	2.8	3.5

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.

TABLE 16. CONCENTRATION OF TETRACYCLINE IN BONE OF MICE IN THE THIRTEEN-WEEK FEED STUDIES (a)

	Tetracycline Hydrochloride in Feed (ppm) 3,100	12,500	50,000
MALE	157	196	651
FEMALE	--	247	600

(a) Mean micrograms/gram of bone for left femurs of five animals

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Mean body weights of high dose male mice were 9%-15% lower than those of controls throughout most of the study (Table 17 and Figure 7). Mean body weights of low dose male mice were 6%-11% lower than those of the controls from week 3 to the end of the study. Mean body weights of high dose female mice were 8%-10% lower than those of the controls from week 1 to week 9 and 13%-28% lower thereafter. Mean body weights of low dose female mice were 5%-10% lower than those of the controls from week 1 to week 9 and 11%-24% lower thereafter. The average daily feed consumption by low dose and high dose male mice was 98% that by the controls and by low dose and high dose female mice 95% and 100% that by the controls (Tables G3 and G4). The average amount of tetracycline hydrochloride consumed per day was approximately 1,500 or 3,000 mg/kg for low dose or high dose male mice and 1,500 or 3,500 mg/kg for low dose or high dose female mice. No compound-related clinical signs were observed.

TABLE 17. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

Weeks on Study	Control		12,500 ppm			25,000 ppm		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE								
0	21.7	50	22.0	101	50	21.6	100	50
1	23.4	50	22.7	97	50	22.1	94	50
2	24.9	50	23.9	96	50	23.2	93	50
3	25.4	50	24.0	94	50	23.5	93	50
4	26.6	50	25.1	94	50	24.7	93	50
5	27.3	50	24.9	91	50	24.3	89	50
6	28.6	50	26.5	93	50	25.4	89	50
7	30.4	50	28.6	94	50	27.7	91	50
8	29.9	50	27.7	93	50	27.3	91	50
9	30.3	50	27.9	92	50	27.6	91	50
10	30.3	50	27.8	92	50	27.2	90	50
11	31.8	50	29.2	92	50	28.4	89	50
12	32.2	50	29.3	91	50	28.6	89	50
13	32.5	50	29.7	91	50	28.8	89	50
17	32.6	49	30.2	93	50	29.8	91	50
22	34.0	49	31.5	93	50	30.6	90	50
26	35.5	49	32.3	91	49	31.5	89	50
31	36.5	48	32.5	89	49	31.9	87	50
35	35.8	48	32.5	91	49	32.0	89	50
39	37.9	48	34.1	90	49	32.4	85	50
43	37.5	48	33.9	90	49	32.3	86	50
48	37.9	48	34.3	91	49	33.2	88	50
52	37.6	48	34.3	91	49	33.2	88	50
57	37.9	48	34.2	90	49	33.1	87	50
61	39.1	47	35.2	90	49	34.4	88	50
65	38.3	47	34.0	89	49	34.1	89	49
69	37.5	46	34.3	91	49	33.5	89	49
74	37.6	45	34.4	91	49	33.8	90	49
78	38.2	45	35.4	93	49	34.3	90	49
82	37.5	43	34.8	93	49	33.5	89	49
86	37.5	39	34.3	91	48	33.6	90	48
91	38.2	37	34.7	91	47	34.0	89	46
95	37.5	37	34.2	91	47	32.8	87	45
99	38.4	34	34.6	90	45	33.8	88	44
104	38.2	31	34.8	91	43	34.0	89	43
FEMALE								
0	19.9	50	19.8	99	50	19.6	98	50
1	20.8	50	19.1	92	50	19.0	91	50
2	21.7	50	20.6	95	50	20.0	92	50
3	22.4	50	21.0	94	50	20.4	91	50
4	23.0	50	21.7	94	50	20.8	90	50
5	23.3	50	21.7	93	50	21.4	92	50
6	24.0	50	22.3	93	50	22.0	92	50
7	25.2	50	23.2	92	50	22.9	91	50
8	24.6	50	22.8	93	50	22.5	91	50
9	25.4	50	22.9	90	50	22.8	90	50
10	26.2	50	23.2	89	50	22.8	87	50
11	27.1	50	24.1	89	50	23.5	87	50
12	27.4	50	24.2	88	50	23.5	86	50
13	28.1	50	24.4	87	50	23.6	84	50
17	29.6	50	25.3	85	50	24.1	81	50
22	31.3	50	26.6	85	50	25.2	81	50
26	33.0	50	26.8	81	50	25.4	77	50
31	33.2	50	27.3	82	50	26.2	79	50
35	35.3	50	28.9	82	49	26.7	76	50
39	36.6	50	29.0	79	48	27.1	74	50
43	36.4	50	28.3	78	48	26.5	73	50
48	36.6	50	28.1	77	47	26.3	72	50
52	35.9	50	28.7	80	47	26.6	74	50
57	36.2	50	29.7	82	46	27.7	77	50
61	37.2	49	29.4	79	46	27.8	75	49
65	37.1	49	30.1	81	46	28.2	76	48
69	36.5	48	30.2	83	46	27.6	76	48
74	37.5	48	31.0	83	45	27.8	74	48
78	37.2	48	30.8	83	45	28.2	76	48
82	37.2	48	30.9	83	45	28.2	76	46
86	38.8	46	31.6	81	45	29.0	75	45
91	39.5	46	31.6	80	43	28.6	72	45
95	40.0	46	30.4	76	42	28.7	72	43
99	39.5	44	30.7	78	41	29.3	74	42
104	38.2	37	32.6	85	34	30.4	80	38

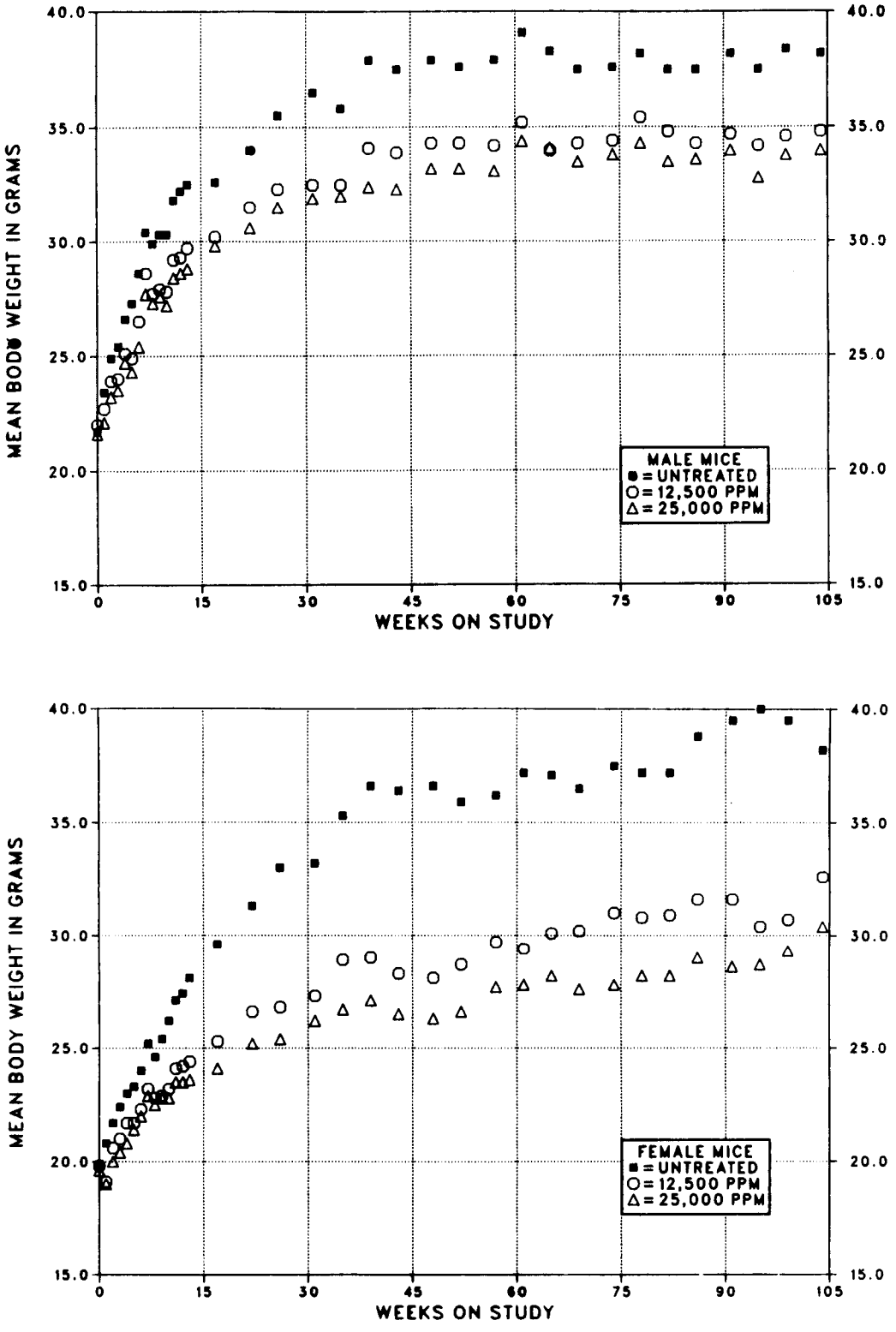

FIGURE 7. GROWTH CURVES FOR MICE FED DIETS CONTAINING TETRACYCLINE HYDROCHLORIDE FOR TWO YEARS

III. RESULTS: MICE

Survival

Estimates of the probabilities of survival for male and female mice fed diets containing tetracycline hydrochloride at the concentrations used in these studies and for controls are shown in Table 18 and in the Kaplan and Meier curves in Figure 8. After week 83, the survival of the male mouse control group was significantly lower than that of both the low and high dose groups. No significant differences in survival were observed between any groups of female mice.

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 thyroid gland, hematopoietic system, liver, anterior pituitary gland, and harderian gland.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors 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 18. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

	Control	12,500 ppm	25,000 ppm
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	19	7	6
Animals missing	0	0	1
Animals surviving until study termination	31	43	43
Survival P values (c)	0.002	0.009	0.005
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	12	13	12
Accidentally killed	0	1	0
Animals missing	1	1	0
Died during termination period	0	1	0
Animals surviving until study termination	37	34	38
Survival P values (c)	1.000	0.884	0.954

(a) Termination period: week 104
(b) Includes animals killed in a moribund condition
(c) 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.

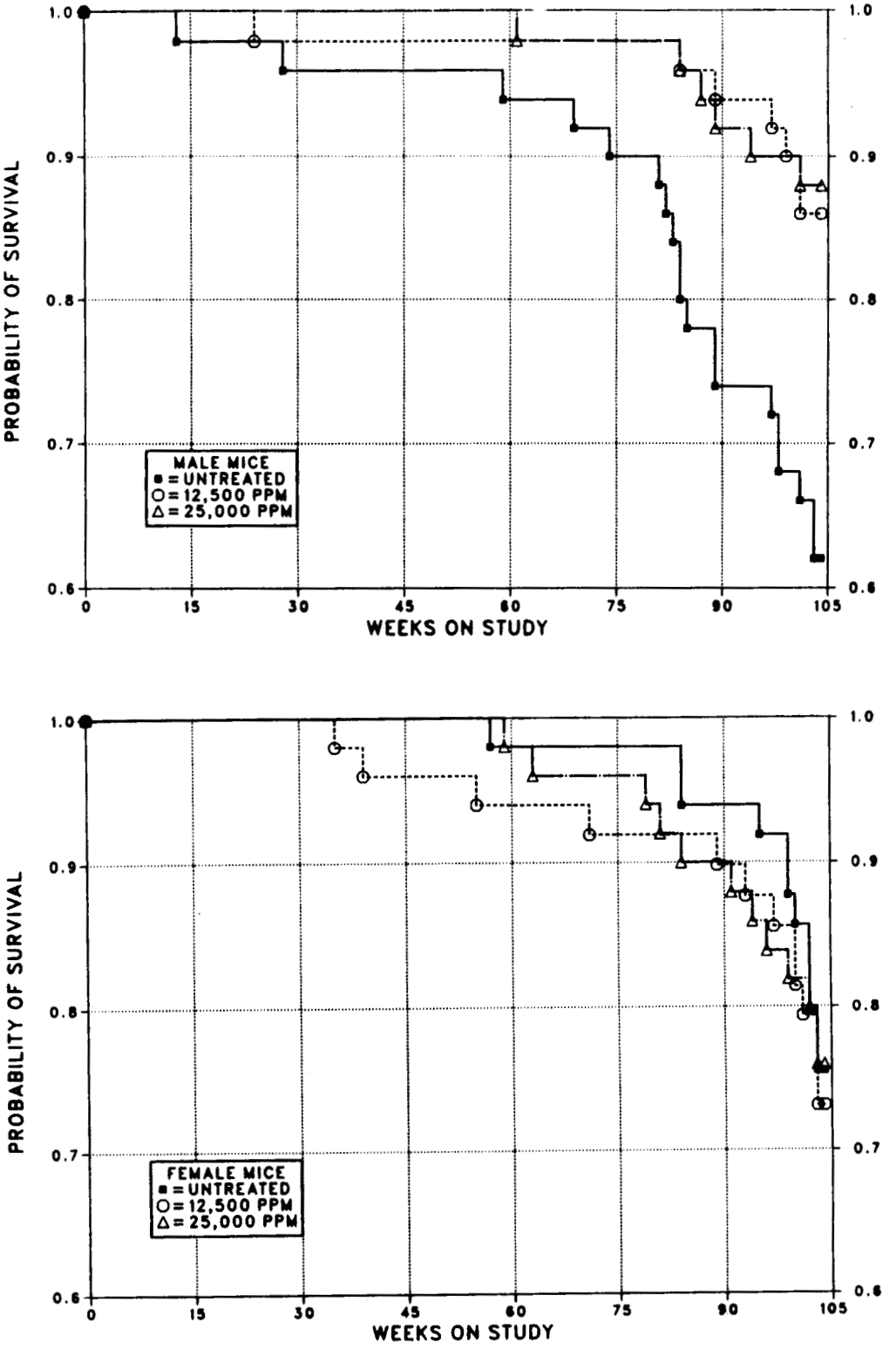

FIGURE 8. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING TETRACYCLINE HYDROCHLORIDE FOR TWO YEARS

Thyroid Gland: Cystic follicles were observed at an increased (P=0.035) incidence in high dose female mice (male: control, 2/49; low dose, 0/3; high dose, 5/47; female: 1/49; 1/47; 7/50). Follicular cell adenomas in female mice occurred with a significant negative trend (4/49; 0/47; 0/50; P<0.025); the incidences in the dosed groups were not significantly different from that in the controls.

Hematopoietic System: The incidence of lymphomas in high dose male mice was significantly lower than that in controls (Table 19). Incomplete sampling of liver tissue in the low dose group precluded a statistical evaluation of the incidence of malignant lymphomas in this group.

Liver: Hepatocellular adenomas and hepatocellular adenomas or carcinomas (combined) in female mice occurred with significant negative trends; the incidences in the dosed groups were significantly lower than those in the controls (Table 20).

TABLE 19. MALIGNANT LYMPHOMAS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (a,b)

	Control	12,500 ppm (c)	25,000 ppm (c)
Overall Rates	9/49 (18%)	(d) 3/50 (6%)	2/48 (4%)
Adjusted Rates	24.1%		4.7%
Terminal Rates	5/31 (16%)		2/43 (5%)
Week of First Observation	13		104
Life Table Test			P=0.010N
Incidental Tumor Test			P=0.054N

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table C3 (footnotes).
(b) Historical incidence of lymphomas or leukemia (combined) at study laboratory (mean ± SD): 19/150 (13% ± 6%); historical incidence in NTP studies: 243/2,040 (12% ± 7%)
(c) The estimated dose in milligrams per kilograms per day is given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix G.
(d) Incomplete sampling of tissue for histologic evaluation precluded statistical comparisons

TABLE 20. HEPATOCELLULAR TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Control	12,500 ppm	25,000 ppm
Adenoma			
Overall Rates	8/49 (16%)	0/48 (0%)	0/50 (0%)
Adjusted Rates	20.7%	0.0%	0.0%
Terminal Rates	7/37 (19%)	0/35 (0%)	0/38 (0%)
Week of First Observation	99		
Life Table Tests	P<0.001N	P=0.006N	P=0.005N
Incidental Tumor Tests	P<0.001N	P=0.007N	P=0.006N
Carcinoma			
Overall Rates	3/49 (6%)	0/48 (0%)	0/50 (0%)
Adenoma or Carcinoma (a)			
Overall Rates	10/49 (20%)	0/48 (0%)	0/50 (0%)
Adjusted Rates	25.3%	0.0%	0.0%
Terminal Rates	8/37 (22%)	0/35 (0%)	0/38 (0%)
Week of First Observation	99		
Life Table Tests	P<0.001N	P=0.002N	P=0.001N
Incidental Tumor Tests	P<0.001N	P=0.002N	P=0.002N

(a) Historical incidence at study laboratory (mean ± SD): 18/149 (12% ± 6%); historical incidence in NTP studies: 177/2,033 (9% ± 5%)

Anterior Pituitary Gland: Adenomas and adenomas or carcinomas (combined) in female mice occurred with significant negative trends; the incidences in the low dose group were significantly lower than those in the controls (Table 21).

Harderian Gland: Adenomas occurred in male mice with a significant negative trend (control, 4/49; low dose, 0/50; high dose, 1/48; P=0.041); the incidence in the low dose group was significantly lower than that in the controls (P=0.030).

TABLE 21. ANTERIOR PITUITARY GLAND LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Control	12,500 ppm	25,000 ppm
Focal Hyperplasia			
Overall Rates	11/48 (23%)	16/46 (35%)	7/49 (14%)
Adenoma			
Overall Rates	11/48 (23%)	2/46 (4%)	5/49 (10%)
Adjusted Rates	28.9%	5.9%	12.3%
Terminal Rates	10/37 (27%)	2/34 (6%)	3/38 (8%)
Week of First Observation	103	104	99
Life Table Tests	P=0.045N	P=0.012N	P=0.083N
Incidental Tumor Tests	P=0.056N	P=0.013N	P=0.105N
Carcinoma			
Overall Rates	1/48 (2%)	0/46 (0%)	0/49 (0%)
Adenoma or Carcinoma (a)			
Overall Rates	12/48 (25%)	2/46 (4%)	5/49 (10%)
Adjusted Rates	30.8%	5.9%	12.3%
Terminal Rates	10/37 (27%)	2/34 (6%)	3/38 (8%)
Week of First Observation	103	104	99
Life Table Tests	P=0.026N	P=0.007N	P=0.054N
Incidental Tumor Tests	P=0.035N	P=0.009N	P=0.075N

(a) Historical incidence at study laboratory (mean ± SD): 26/146 (18% ± 16%); historical incidence in NTP studies: 204/1,764 (12% ± 10%)

IV. DISCUSSION AND CONCLUSIONS

IV. DISCUSSION AND CONCLUSIONS

The first tetracycline-like antibiotic, chlortetracycline hydrochloride, was discovered by Duggar in 1948 (Siegel, 1978; Boothe and Hlavka, 1978). Oxytetracycline followed in 1950 and tetracycline in 1953; these two were the first commercially available tetracycline bases to be produced by methods other than fermentation. Although numerous reports have described the therapeutic and toxic effects of tetracycline hydrochloride, adequate and comprehensive lifetime toxicity and carcinogenicity studies in rodents had not been reported. The current studies were conducted because of the widespread use of tetracycline hydrochloride as a broad-spectrum antibiotic in humans and the lack of adequate data from long-term animal studies.

Tetracycline hydrochloride combines with copper, nickel, or cobalt ions to form complexes that bind to nucleic acids (Mikelens and Levinson, 1978). This is one possible mechanism by which the drug, through mRNA complexing and subsequent misarrangements of tRNA, could inhibit protein synthesis within the bacterial target cell (Connamacher and Mandel, 1965). Tetracycline hydrochloride gave positive results in the DNA cell binding assay, which suggests mutagenic potential (Kubinski et al., 1981). However, no positive responses were observed when tetracycline hydrochloride was tested in the presence or absence of metabolic activation for induction of reverse mutations in four strains of Salmonella (Table E1), cytogenetic damage in cultured Chinese hamster ovary cells (Table E3 and E4), or sex-linked recessive lethal mutations in Drosophila (Table E5). The only reports of mutagenic activity by tetracycline hydrochloride concern induction of 8-azaguanine-resistant mutants in cultured mammalian cells and the weak induction of trifluorothymidine resistance in mouse lymphoma cells in the presence of noninduced S9.

Tetracycline hydrochloride (USP grade, 91% pure) was administered in feed to groups of F344/N rats and B6C3F$_1$ mice for 14 days, 13 weeks, and 2 years. The 14-day studies in rats and mice were conducted with tetracycline hydrochloride in feed at concentrations up to 50,000 ppm; no deaths were recorded. Decreased final body weights were limited to the 50,000-ppm male rats and mice and the 25,000- and 50,000-ppm female mice. These groups consumed less feed than did the controls, primarily during the first week of the studies; the male mice also consumed less feed during week 2. No pathologic changes were observed grossly.

During the 13-week studies, tetracycline ingestion at all dose levels in both species did not cause deaths. Male rats and mice in the top dose groups (50,000 ppm) had lower body weight gains than did their controls. These body weight changes were not associated with drug-induced changes in feed consumption; feed consumption by dosed groups was comparable to that by controls in all cases. No compound-related histologic changes were noted in mice. In rats, cytoplasmic vacuolization of the liver occurred in males receiving 50,000 ppm (10/10) and 25,000 ppm (9/10) tetracycline hydrochloride. Short-term parenteral administration of tetracycline to rats at high doses has been reported to induce fatty livers and vacuolization (Steiner et al., 1965; Breen et al., 1975; Bernheim, 1971; Sande and Mandell, 1985). Bone levels of the antibiotic increased with increased concentrations of the drug in the diet of both rats and mice.

Dietary concentrations of 0, 12,500, and 25,000 ppm tetracycline hydrochloride were selected for the 2-year studies in rats and mice. These exposure concentrations represent approximately 20-fold to 45-fold increases for rats and 70-fold to 150-fold increases for mice compared with daily therapeutic human doses expressed as milligrams of tetracycline hydrochloride per kilogram body weight per day (Sande and Mandell, 1985; PDR, 1987; Appendix G).

Body weights and feed consumption values for male and female rats were not affected by tetracycline consumption during the 2-year studies. Estimated daily tetracycline hydrochloride consumption for low and high dose rats averaged 440-510 and 910-1,060 mg/kg. Although no significant differences in the survival of male groups were noted, the survival of both the low (after week 95) and high (at the end of the study) dose females was greater than that of controls (see Table 11). In contrast, male and female mice showed tetracycline-induced depressions in body weight. These results were not predicted from the results of the 13-week studies. There

were no drug-associated changes in feed consumption. Estimated daily tetracycline hydrochloride consumption values for low and high dose mice averaged 1,500 and 3,000-3,500 mg/kg. Mean body weights of dosed mice, especially those of the females, were lower than those of controls (see Table 17). Survival of low and high dose male mice was greater than that of controls after week 83.

The average control survival at the end of the studies for rats (54%) and mice (68%) is adequate but less than the average for previous NTP studies (Rao et al., 1987). In other 2-year feed studies, tetracycline consumption increased survival in dosed male Osborne-Mendel rats compared with that in controls (control, 57%; 100 ppm, 60%; 1,000 ppm, 72%; 3,000 ppm, 70%) (Deichmann et al., 1964). This was attributed to the "protective" effect of this antibiotic. An inverse correlation between survival and body weight was noted in male mice in the study described in this report. This correlation was not noted in female mice despite the fact that body weight in this group was depressed to a greater extent than in males. However, no changes in diet consumption were associated with tetracycline hydrochloride administration to rats or mice during the 2-year studies. McKay et al. (1935) first reported that food restriction in rodents extends longevity, and Harrison and Archer (1987) recently showed that diet restriction and consequent lower body weights are associated with increased survival in a variety of mouse strains. Yu et al. (1985) showed that diet restriction also increased the life span of F344/N rats.

During the conduct of the 2-year toxicity and carcinogenicity studies, administration of tetracycline hydrochloride was associated with negative trends in the incidences of naturally occurring neoplasms related to aging, including malignant lymphomas in male mice, anterior pituitary gland adenomas in female mice, hepatocellular adenomas and carcinomas in female mice, mononuclear cell leukemia in male rats, and anterior pituitary gland adenomas in female rats. The incidence of leukemia in control male rats (36/50) in this study is greater than the incidences in untreated animals (25/50; 24/50; 22/50) from the three previous studies at the study laboratory (Table A4b). The incidences of lymphomas (8/50; 3/50; 8/50) in control male mice from previous studies at the study laboratory (Table C4) are similar to that in the present study (9/49). Ten hepatocellular adenomas or carcinomas (combined) occurred in control female mice but none in tetracycline hydrochloride-exposed females. This striking tetracycline-associated negative trend is the only biologically significant neoplastic effect from these studies.

The influence of decreased body weight on tumor incidence is possibly associated with the decrease in liver neoplasia, since both groups of dosed female mice weighed considerably less than did controls. Rao et al. (1987) reported a marginal correlation between liver tumor occurrence and body weights for extreme differences in body weights but did not find an overall association.

In female rats, increased incidences of focal anterior pituitary gland hyperplasia (control, 5/48; low dose, 8/43; high dose, 15/50) and a negative trend for the incidences of anterior pituitary gland adenomas (25/48; 24/43; 18/50) were noted. Since these are considered part of a neoplastic progression (Ward, 1984), neither lesion is considered to be associated with administration of tetracycline hydrochloride; i.e., adding these incidences cancels any apparent trend. Tetracycline hydrochloride did not increase the incidence of hyperplasia in female mice; a decrease in the incidences of anterior pituitary gland adenomas was observed in dosed female mice. Female mice, however, showed a drug-related decrease in body weight, which has been shown to be associated with a decrease in various neoplasms, including those of the pituitary gland (Ross and Bras, 1973; Ross et al., 1983).

Administration of tetracycline was associated with decreased incidences (control, 48/50; low dose, 35/50; high dose, 36/50) and severity (control, 2.8; low dose, 1.6; high dose, 1.5) of chronic nephropathy in male rats. The literature on effects in humans indicates that tetracycline treatment normally exacerbates preexisting renal lesions (Bernheim, 1971; Sande and Mandell, 1985).

Other negative trends associated with tetracycline hydrochloride administration involved the bile duct, pancreas, adrenal gland, mammary gland, and thyroid gland in rats and the harderian gland and thyroid gland in mice. These changes were either not statistically significant or were considered to be of questionable biologic significance.

Exposure to tetracycline hydrochloride increased the incidences of hepatic and pancreatic islet cell lesions in rats. Tetracycline hydrochloride induced dose-related increases in the incidences of basophilic cytologic changes and clear cell changes in male rat liver. Female rat liver, on the other hand, was unaffected by administration of tetracycline hydrochloride. The cytotoxic changes noted in the liver of males were not unexpected, since tetracycline given in large parenteral doses has been shown to induce fatty degeneration of rat liver (Seto and Lepper, 1954; Lewis et al., 1967; Zussman, 1968; Damjanov and Solter, 1970; De Jonge, 1973; Breen et al., 1975). Lepper et al. (1951) showed that hepatic changes (lipid vacuolation) induced in mice by chlortetracycline were more marked and required less drug after parenteral exposure compared with changes induced by chlortetracycline given orally. In the current studies, low dose male rats developed pancreatic islet cell neoplasms (adenomas and carcinomas) at a higher rate than did controls and high dose male rats, which did not develop these neoplasms. The percentage of low dose males developing these neoplasms was 10% (5/49) compared with control incidences of 1/50, 2/48, and 6/50 in other studies conducted at the same laboratory (Table A4a). The marginal increase of these neoplasms in low dose males was not considered to be chemically related.

Toxicology and carcinogenesis studies of a related tetracycline, oxytetracycline hydrochloride, were conducted by the NTP (NTP, 1987). In those studies, rats and mice received 0, 25,000, or 50,000 ppm oxytetracycline hydrochloride in the diet for 2 years. Pheochromocytomas of the adrenal gland occurred with a positive trend in male rats, and adenomas and adenomas or adenocarcinomas (combined) of the anterior pituitary gland occurred with positive trends in female rats. These results were considered equivocal evidence of carcinogenicity for male and female F344/N rats. In the current studies with tetracycline hydrochloride, no increases in tumor incidence were seen in the adrenal or pituitary glands in rats.

The experimental and tabulated data for the NTP Technical Report on tetracycline hydrochloride were examined for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations. As summarized in Appendix I, 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 feed studies, there was *no evidence of carcinogenic activity** of tetracycline hydrochloride for male or female F344/N rats and B6C3F$_1$ mice fed diets containing 12,500 or 25,000 ppm. Tetracycline hydrochloride-dosed female rats and male mice had greater survival rates than the respective controls during these studies. Dosed mice had lower body weight than controls, and dosed female mice had no hepatocellular adenomas or carcinomas.

*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

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals necropsied	50	50	50
Animals examined histologically	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Basal cell tumor		1 (2%)	1 (2%)
Trichoepithelioma		1 (2%)	1 (2%)
Sebaceous adenoma			1 (2%)
Keratoacanthoma	1 (2%)	2 (4%)	
*Subcutaneous tissue	(50)	(50)	(50)
Fibroma	3 (6%)	2 (4%)	1 (2%)
Fibrosarcoma		1 (2%)	1 (2%)
Lipoma		1 (2%)	1 (2%)
Neurilemoma, malignant			1 (2%)
RESPIRATORY SYSTEM			
#Nasal turbinate	(50)	(11)	(49)
Fibrosarcoma		1 (9%)	
#Lung	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic			1 (2%)
Alveolar/bronchiolar adenoma			2 (4%)
C-cell carcinoma, metastatic	2 (4%)		
Pheochromocytoma, metastatic		1 (2%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukemia, mononuclear cell	36 (72%)	39 (78%)	24 (48%)
#Bone marrow	(50)	(19)	(49)
Pheochromocytoma, metastatic			1 (2%)
Osteosarcoma			1 (2%)
CIRCULATORY SYSTEM			
#Heart	(50)	(21)	(50)
Neurilemoma	1 (2%)		
DIGESTIVE SYSTEM			
#Salivary gland	(50)	(15)	(49)
Carcinoma, NOS			1 (2%)
#Liver	(50)	(49)	(50)
Neoplastic nodule		1 (2%)	2 (4%)
Hepatocellular carcinoma		1 (2%)	1 (2%)
Lipoma	1 (2%)		
URINARY SYSTEM			
None			
ENDOCRINE SYSTEM			
#Pituitary intermedia	(48)	(49)	(50)
Adenoma, NOS	1 (2%)		
#Anterior pituitary	(48)	(49)	(50)
Adenoma, NOS	12 (25%)	17 (35%)	10 (20%)
#Adrenal medulla	(50)	(50)	(49)
Pheochromocytoma	21 (42%)	18 (36%)	14 (29%)
Pheochromocytoma, malignant		3 (6%)	2 (4%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Thyroid	(50)	(14)	(50)
C-cell adenoma	6 (12%)	2 (14%)	4 (8%)
C-cell carcinoma	4 (8%)	1 (7%)	4 (8%)
#Parathyroid	(39)	(10)	(34)
Adenoma, NOS			1 (3%)
#Pancreatic islets	(49)	(49)	(49)
Islet cell adenoma		3 (6%)	
Islet cell carcinoma		2 (4%)	
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Fibroadenoma	1 (2%)		2 (4%)
*Preputial gland	(50)	(50)	(50)
Adenoma, NOS	5 (10%)	4 (8%)	6 (12%)
#Testis	(50)	(40)	(49)
Interstitial cell tumor	44 (88%)	31 (78%)	41 (84%)
NERVOUS SYSTEM			
#Cerebrum	(50)	(13)	(50)
Astrocytoma			1 (2%)
*Spinal cord	(50)	(50)	(50)
Glioma, NOS			1 (2%)
SPECIAL SENSE ORGANS			
*Zymbal gland	(50)	(50)	(50)
Carcinoma, NOS		1 (2%)	
C-cell carcinoma, invasive	1 (2%)		
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
*Tunica vaginalis	(50)	(50)	(50)
Mesothelioma, NOS		1 (2%)	
Mesothelioma, malignant	1 (2%)		
ALL OTHER SYSTEMS			
None			
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	2	7	6
Moribund sacrifice	21	19	13
Terminal sacrifice	27	24	31

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	49	50	49
Total primary tumors	137	133	124
Total animals with benign tumors	48	47	46
Total benign tumors	96	82	85
Total animals with malignant tumors	37	42	33
Total malignant tumors	41	49	37
Total animals with secondary tumors##	2	1	2
Total secondary tumors	3	1	2
Total animals with tumors uncertain-- benign or malignant		2	2
Total uncertain tumors		2	2

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

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: UNTREATED CONTROL

ANIMAL NUMBER	112	111	140	103	128	148	106	116	117	129	144	113	102	101	146	141	109	122	104	110	120	127	150	105	107
WEEKS ON STUDY	051	057	067	074	079	082	085	085	088	089	090	091	094	096	097	098	100	100	101	101	101	102	102	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma															X			X	X						
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell carcinoma, metastatic											X									X					
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neurilemoma																									
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma								X																	
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS						X						X				X	X		X	X			X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma							X	X		X					X	X		X	X			X	X	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma																	X	X	X						
C-cell carcinoma											X									X	X				
Parathyroid	−	+	−	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	−	−	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																							X		
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor				X	X	X	X	X	X	X	X		X	X	X	X	X	X		X	X	X		X	X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS			X												X			X							
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N
C-cell carcinoma, invasive																				X					
BODY CAVITIES																									
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, malignant																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell	X				X		X	X	X		X		X	X	X	X	X	X	X		X	X	X	X	X

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
*: Animals necropsied
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

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

ANIMAL NUMBER	108	114	115	118	119	121	123	124	125	128	130	131	132	133	134	135	136	137	138	139	142	143	145	147	149	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Keratoacanthoma																	X									1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																										3
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell carcinoma, metastatic																										2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neurilemoma			X																							1
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lipoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma, NOS				X		X			X	X						X					X					13
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma	X		X			X	X		X			X	X	X				X			X			X	X	21
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma		X								X			X													6
C-cell carcinoma									X																	4
Parathyroid	+	+	+	+	−	−	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	39
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroadenoma																										1
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	44
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	50
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS									X		X															5
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
C-cell carcinoma, invasive																										1
BODY CAVITIES																										
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Mesothelioma, malignant																									X	1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell	X	X	X	X	X		X		X		X		X	X	X			X		X	X	X	X	X	X	36

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: LOW DOSE

ANIMAL NUMBER	0115	0118	0228	0422	0449	0229	0223	0337	0339	0440	0338	0009	0433	0333	0441	0002	0004	0116	0008	0335	0446	0005	0119	0332	0334
WEEKS ON STUDY	074	079	079	084	084	085	088	089	089	089	090	093	093	094	094	096	096	096	099	099	099	102	102	102	102
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	N	N	N	N	N	N	N	N	+	+	N	N	N	N
Basal cell tumor																									
Trichoepithelioma	X																								
Keratoacanthoma																					X				
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	N	N	N	N	N	N	N	N	+	+	N	N	N	N
Fibroma					X																				
Fibrosarcoma																									
Lipoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma, metastatic																					X				
Trachea	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fibrosarcoma											X														
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–	–	–	+	+	–	–	+	–	–
Thymus	+	+	+	+	+	+	–	+	+	+	+	–	–	+		–	–	–	–	–	–	–	–	–	–
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–	–	–	+	–	+	+	+	–	–
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Neoplastic nodule																									
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Stomach	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Small intestine	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Large intestine	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ENDOCRINE SYSTEM																									
Pituitary	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS						X			X	X						X	X					X			X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma				X			X	X					X						X	X				X	
Pheochromocytoma, malignant																					X			X	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C cell adenoma										X															
C cell carcinoma																									
Parathyroid	+	+	+	+	+	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
Islet cell carcinoma			X																			X			
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	–	–	–	–	+	+	+
Interstitial cell tumor				X			X	X	X		X	X			X		X	X					X	X	X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS					X																				
BODY CAVITIES																									
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+	+	N	N	N	N	+	+	+
Mesothelioma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell	X	X		X	X	X	X	X	X	X		X		X	X	X		X	X	X	X		X	X	X

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	050	001	003	006	007	010	011	012	013	014	017	020	021	022	024	025	026	027	030	031	036	044	045	047	048	TOTAL:
WEEKS ON STUDY	102	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	N	N	N	N	N	N	N	N	+	+	+	N	N	+	N	N	N	N	+	N	N	N	N	N	*50
Basal cell tumor												X														1
Trichoepithelioma																										1
Keratoacanthoma																				X						2
Subcutaneous tissue	+	N	N	N	N	N	N	N	N	+	+	+	N	N	+	N	N	N	N	+	N	N	N	N	N	*50
Fibroma											X															2
Fibrosarcoma										X																1
Lipoma															X											1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma, metastatic																										1
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	14
Nasal cavity	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11
Fibrosarcoma																										1
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	+	–	–	+	–	–	–	+	–	+	–	–	–	–	–	+	–	–	–	–	–	–	19
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph nodes	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	–	–	–	+	–	+	–	21
Thymus	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–	14
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	+	+	–	–	–	+	–	–	–	21
DIGESTIVE SYSTEM																										
Salivary gland	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	15
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Neoplastic nodule														X												1
Hepatocellular carcinoma																								X		1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	49
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	13
Stomach	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12
Small intestine	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12
Large intestine	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS				X					X				X	X	X	X			X			X	X	X		17
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma			X		X			X		X	X					X		X	X			X		X	X	18
Pheochromocytoma, malignant																		X								3
Thyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	14
C-cell adenoma																								X		2
C-cell carcinoma																							X			1
Parathyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	10
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	49
Islet cell adenoma																	X		X				X			3
Islet cell carcinoma																										2
REPRODUCTIVE SYSTEM																										
Mammary gland	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	–	–	+	+	40
Interstitial cell tumor	X	X	X	X	X	X	X	X		X	X	X	X	X			X	X		X	X			X	X	31
Prostate	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS		X	X												X							X				4
NERVOUS SYSTEM																										
Brain	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	13
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
BODY CAVITIES																										
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+	+	N	N	+	+	*50
Mesothelioma, NOS																									X	1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell	X	X	X			X			X	X	X	X	X	X	X		X	X		X	X	X	X	X	X	39

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: HIGH DOSE

ANIMAL NUMBER	072	080	066	067	056	055	075	073	095	068	090	061	088	053	054	057	092	063	097	051	052	058	059	060	062
WEEKS ON STUDY	037	064	076	077	082	088	088	090	093	096	096	098	099	101	101	102	102	103	103	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell tumor																									
Trichoepithelioma														X											
Sebaceous adenoma															X										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
Fibrosarcoma																									
Lipoma																			X						
Neurilemoma, malignant														X											
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic							X																		
Alveolar/bronchiolar adenoma																X									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma, metastatic																									
Osteosarcoma																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Hepatocellular carcinoma							X																		
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS					X	X											X	X					X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma											X		X										X	X	
Pheochromocytoma, malignant												X													
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma												X													
C-cell carcinoma						X												X							
Parathyroid	+	−	−	+	+	+	+	−	+	−	+	+	+	+	+	−	+	−	+	+	+	−	+	+	+
Adenoma, NOS																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																	X								
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Interstitial cell tumor			X				X	X		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																				X					
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma																									
Spinal cord	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Glioma, NOS		X																							
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell			X	X		X		X	X	X	X	X	X		X	X		X	X	X		X			

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	064	065	069	070	071	074	076	077	078	079	081	082	083	084	085	086	087	089	091	093	094	096	098	099	100	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Basal cell tumor																	X									1
Trichoepithelioma																										1
Sebaceous adenoma																										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																						X				1
Fibrosarcoma																									X	1
Lipoma																										1
Neurilemoma, malignant																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic																										1
Alveolar/bronchiolar adenoma																									X	2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma, metastatic					X																					1
Osteosarcoma								X																		1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	49
Carcinoma, NOS																				X						1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule						X													X							2
Hepatocellular carcinoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS					X									X			X		X						X	10
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma			X			X			X			X		X		X		X		X	X	X				14
Pheochromocytoma, malignant					X																					2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma						X				X												X				4
C-cell carcinoma			X																					X		4
Parathyroid	+	+	+	+	−	+	+	+	−	+	+	−	−	+	−	−	−	−	+	−	+	+	+	+	+	34
Adenoma, NOS											X															1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroadenoma																		X								2
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	41
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS												X	X						X			X			X	6
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma																					X					1
Spinal cord	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Glioma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell		X		X		X			X						X	X		X			X		X		24

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Control	12,500 ppm	25,000 ppm
Skin: Trichoepithelioma, Basal Cell Tumor, or Sebaceous Adenoma			
Overall Rates (a)	0/50 (0%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	0.0%	6.1%	8.5%
Terminal Rates (c)	0/27 (0%)	1/24 (4%)	1/31 (3%)
Week of First Observation		74	101
Life Table Tests (d)	P=0.108	P=0.233	P=0.149
Incidental Tumor Tests (d)	P=0.096	P=0.119	P=0.128
Cochran-Armitage Trend Test (d)	P=0.082		
Fisher Exact Test (d)		P=0.247	P=0.121
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	8.6%	6.2%	3.2%
Terminal Rates (c)	0/27 (0%)	1/24 (4%)	1/31 (3%)
Week of First Observation	97	84	104
Life Table Tests (d)	P=0.200N	P=0.538N	P=0.270N
Incidental Tumor Tests (d)	P=0.227N	P=0.406N	P=0.289N
Cochran-Armitage Trend Test (d)	P=0.222N		
Fisher Exact Test (d)		P=0.500N	P=0.309N
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted Rates (b)	8.6%	10.3%	6.5%
Terminal Rates (c)	0/27 (0%)	2/24 (8%)	2/31 (6%)
Week of First Observation	97	84	104
Life Table Tests (d)	P=0.364N	P=0.617	P=0.448N
Incidental Tumor Tests (d)	P=0.408N	P=0.591N	P=0.475N
Cochran-Armitage Trend Test (d)	P=0.412N		
Fisher Exact Test (d)		P=0.661	P=0.500N
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	36/50 (72%)	39/50 (78%)	24/50 (48%)
Adjusted Rates (b)	83.4%	86.1%	53.8%
Terminal Rates (c)	20/27 (74%)	18/24 (75%)	11/31 (35%)
Week of First Observation	51	74	76
Life Table Tests (d)	P=0.012N	P=0.254	P=0.013N
Incidental Tumor Tests (d)	P=0.009N	P=0.415	P=0.012N
Cochran-Armitage Trend Test (d)	P=0.008N		
Fisher Exact Test (d)		P=0.322	P=0.012N
Liver: Neoplastic Nodule or Hepatocellular Carcinoma			
Overall Rates (a)	0/50 (0%)	2/49 (4%)	3/50 (6%)
Adjusted Rates (b)	0.0%	8.3%	8.5%
Terminal Rates (c)	0/27 (0%)	2/24 (8%)	2/31 (6%)
Week of First Observation		104	88
Life Table Tests (d)	P=0.107	P=0.212	P=0.144
Incidental Tumor Tests (d)	P=0.083	P=0.212	P=0.097
Cochran-Armitage Trend Test (d)	P=0.083		
Fisher Exact Test (d)		P=0.242	P=0.121
Anterior Pituitary Gland: Adenoma			
Overall Rates (a)	12/48 (25%)	17/49 (35%)	10/50 (20%)
Adjusted Rates (b)	34.3%	52.3%	27.3%
Terminal Rates (c)	5/26 (19%)	10/24 (42%)	6/31 (19%)
Week of First Observation	82	85	82
Life Table Test (d)	P=0.236N	P=0.166	P=0.281N
Incidental Tumor Test (d)	P=0.341N	P=0.283	P=0.415N
Cochran-Armitage Trend Test (d)	P=0.321N		
Fisher Exact Test (d)		P=0.206	P=0.363N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Control	12,500 ppm	25,000 ppm
Adrenal Medulla: Pheochromocytoma			
Overall Rates (a)	21/50 (42%)	18/50 (36%)	14/49 (29%)
Adjusted Rates (b)	57.2%	55.4%	43.0%
Terminal Rates (c)	12/27 (44%)	11/24 (46%)	12/30 (40%)
Week of First Observation	85	84	96
Life Table Tests (d)	P=0.051N	P=0.462N	P=0.059N
Incidental Tumor Tests (d)	P=0.074N	P=0.277N	P=0.089N
Cochran-Armitage Trend Test (d)	P=0.099N		
Fisher Exact Test (d)		P=0.341N	P=0.118N
Adrenal Medulla: Malignant Pheochromocytoma			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	2/49 (4%)
Adjusted Rates (b)	0.0%	10.4%	5.8%
Terminal Rates (c)	0/27 (0%)	1/24 (4%)	1/30 (3%)
Week of First Observation		99	98
Life Table Tests (d)	P=0.245	P=0.114	P=0.264
Incidental Tumor Tests (d)	P=0.208	P=0.151	P=0.251
Cochran-Armitage Trend Test (d)	P=0.196		
Fisher Exact Test (d)		P=0.121	P=0.242
Adrenal Medulla: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (a)	21/50 (42%)	19/50 (38%)	16/49 (33%)
Adjusted Rates (b)	57.2%	56.9%	47.6%
Terminal Rates (c)	12/27 (44%)	11/24 (46%)	13/30 (43%)
Week of First Observation	85	84	96
Life Table Tests (d)	P=0.108N	P=0.538N	P=0.124N
Incidental Tumor Tests (d)	P=0.155N	P=0.339N	P=0.179N
Cochran-Armitage Trend Test (d)	P=0.196N		
Fisher Exact Test (d)		P=0.419N	P=0.226N
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	6/50 (12%)	(e) 2/14 (14%)	4/50 (8%)
Adjusted Rates (b)	18.7%		12.0%
Terminal Rates (c)	3/27 (11%)		3/31 (10%)
Week of First Observation	98		98
Life Table Test (d)			P=0.300N
Incidental Tumor Test (d)			P=0.318N
Fisher Exact Test (d)			P=0.371N
Thyroid Gland: C-Cell Carcinoma			
Overall Rates (a)	4/50 (8%)	(e) 1/14 (7%)	4/50 (8%)
Adjusted Rates (b)	12.0%		11.3%
Terminal Rates (c)	1/27 (4%)		2/31 (6%)
Week of First Observation	90		88
Life Table Test (d)			P=0.568N
Incidental Tumor Test (d)			P=0.580
Fisher Exact Test (d)			P=0.643N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	10/50 (20%)	(e) 3/14 (21%)	8/50 (16%)
Adjusted Rates (b)	28.8%		22.5%
Terminal Rates (c)	4/27 (15%)		5/31 (16%)
Week of First Observation	90		88
Life Table Test (d)			P=0.301N
Incidental Tumor Test (d)			P=0.400N
Fisher Exact Test (d)			P=0.398N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Control	12,500 ppm	25,000 ppm
Pancreatic Islets: Islet Cell Adenoma			
Overall Rates (a)	0/49 (0%)	3/49 (6%)	0/49 (0%)
Adjusted Rates (b)	0.0%	13.0%	0.0%
Terminal Rates (c)	0/27 (0%)	3/23 (13%)	0/31 (0%)
Week of First Observation		104	
Life Table Tests (d)	P=0.596N	P=0.093	(f)
Incidental Tumor Tests (d)	P=0.596N	P=0.093	(f)
Cochran-Armitage Trend Test (d)	P=0.640		
Fisher Exact Test (d)		P=0.121	(f)
Pancreatic Islets: Islet Cell Adenoma or Carcinoma			
Overall Rates (a)	0/49 (0%)	5/49 (10%)	0/49 (0%)
Adjusted Rates (b)	0.0%	17.8%	0.0%
Terminal Rates (c)	0/27 (0%)	3/23 (13%)	0/31 (0%)
Week of First Observation		79	
Life Table Tests (d)	P=0.562N	P=0.028	(f)
Incidental Tumor Tests (d)	P=0.609	P=0.037	(f)
Cochran-Armitage Trend Test (d)	P=0.610		
Fisher Exact Test (d)		P=0.028	(f)
Preputial Gland: Adenoma			
Overall Rates (a)	5/50 (10%)	4/50 (8%)	6/50 (12%)
Adjusted Rates (b)	14.4%	16.7%	19.4%
Terminal Rates (c)	2/27 (7%)	4/24 (17%)	6/31 (19%)
Week of First Observation	67	104	104
Life Table Tests (d)	P=0.519	P=0.565N	P=0.580
Incidental Tumor Tests (d)	P=0.511	P=0.576N	P=0.570
Cochran-Armitage Trend Test (d)	P=0.434		
Fisher Exact Test (d)		P=0.500N	P=0.500
Testis: Interstitial Cell Tumor			
Overall Rates (a)	44/50 (88%)	31/40 (78%)	41/49 (84%)
Adjusted Rates (b)	100.0%	93.2%	95.3%
Terminal Rates (c)	27/27 (100%)	18/20 (90%)	29/31 (94%)
Week of First Observation	74	84	76
Life Table Tests (d)	P=0.082N	P=0.188N	P=0.095N
Incidental Tumor Tests (d)	P=0.175N	P=0.075N	P=0.245N
Cochran-Armitage Trend Test (d)	P=0.326N		
Fisher Exact Test (d)		P=0.149N	P=0.371N
All Sites: Benign Tumors			
Overall Rates (a)	48/50 (96%)	47/50 (94%)	46/50 (92%)
Adjusted Rates (b)	100.0%	100.0%	100.0%
Terminal Rates (c)	27/27 (100%)	24/24 (100%)	31/31 (100%)
Week of First Observation	67	74	76
Life Table Tests (d)	P=0.116N	P=0.425	P=0.128N
Incidental Tumor Tests (d)	P=0.171N	P=0.276N	P=0.275N
Cochran-Armitage Trend Test (d)	P=0.264N		
Fisher Exact Test (d)		P=0.500N	P=0.339N
All Sites: Malignant Tumors			
Overall Rates (a)	37/50 (74%)	42/50 (84%)	33/50 (66%)
Adjusted Rates (b)	84.0%	87.3%	69.9%
Terminal Rates (c)	20/27 (74%)	18/24 (75%)	17/31 (55%)
Week of First Observation	51	74	64
Life Table Tests (d)	P=0.130N	P=0.177	P=0.145N
Incidental Tumor Tests (d)	P=0.253N	P=0.270	P=0.259N
Cochran-Armitage Trend Test (d)	P=0.210N		
Fisher Exact Test (d)		P=0.163	P=0.257N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Control	12,500 ppm	25,000 ppm
All Sites: All Tumors			
Overall Rates (a)	49/50 (98%)	50/50 (100%)	49/50 (98%)
Adjusted Rates (b)	100.0%	100.0%	100.0%
Terminal Rates (c)	27/27 (100%)	24/24 (100%)	31/31 (100%)
Week of First Observation	51	74	64
Life Table Tests (d)	P=0.201N	P=0.320	P=0.222N
Incidental Tumor Tests (d)	P=0.643	P=0.718	P=0.760
Cochran-Armitage Trend Test (d)	P=0.669		
Fisher Exact Test (d)		P=0.500	P=0.753N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(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 lower incidence in a dosed group is indicated by (N).
(e) Incomplete sampling of tissues
(f) No P value is reported because no tumors were observed in the 25,000-ppm and control groups.

TABLE A4a. HISTORICAL INCIDENCE OF PANCREATIC ISLET CELL TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Physiological Research Laboratories			
Ephedrine sulfate	1/50	0/50	1/50
Phenylephrine hydrochloride	2/48	0/48	2/48
Oxytetracycline hydrochloride	2/50	4/50	6/50
TOTAL	5/148 (3.4%)	4/148 (2.7%)	9/148 (6.1%)
SD (b)	1.21%	4.62%	5.26%)
Range (c)			
High	2/48	4/50	6/50
Low	1/50	0/50	1/50
Overall Historical Incidence			
TOTAL	64/1,871 (3.4%)	37/1,871 (2.0%)	100/1,871 (5.3%)
SD (b)	3.31%	2.56%	3.61%
Range (c)			
High	6/49	4/49	7/49
Low	0/50	0/50	0/50

(a) Data as of August 7, 1986, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE A4b. HISTORICAL INCIDENCE OF LEUKEMIA IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls
Historical Incidence at Physiological Research Laboratories	
Ephedrine sulfate	25/50
Phenylephrine hydrochloride	24/50
Oxytetracycline hydrochloride	22/50
TOTAL	71/150 (47.3%)
SD (b)	3.06%
Range (c)	
High	25/50
Low	22/50
Overall Historical Incidence	
TOTAL	586/1,937 (30.3%)
SD (b)	11.97%
Range (c)	
High	30/50
Low	5/50

(a) Data as of August 7, 1986, for studies of at least 104 weeks
(b) Standard deviation
(c) 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 FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals necropsied	50	50	50
Animals examined histopathologically	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Epidermal inclusion cyst	1 (2%)	1 (2%)	3 (6%)
Scar			1 (2%)
Hyperkeratosis	1 (2%)		
Acanthosis	2 (4%)		
RESPIRATORY SYSTEM			
#Nasal cavity	(50)	(11)	(49)
Inflammation, acute/chronic			1 (2%)
#Nasal turbinate	(50)	(11)	(49)
Inflammation, acute		1 (9%)	1 (2%)
Inflammation, chronic focal	24 (48%)	5 (45%)	30 (61%)
#Lung	(50)	(50)	(50)
Inflammation, chronic focal	3 (6%)	4 (8%)	3 (6%)
Calcification, focal	1 (2%)		1 (2%)
Alveolar macrophages			1 (2%)
Bronchiolization		2 (4%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(50)	(19)	(49)
Hemorrhage			1 (2%)
Hypoplasia, NOS			2 (4%)
Hyperplasia, NOS	1 (2%)	1 (5%)	5 (10%)
Hyperplasia, reticulum cell			1 (2%)
#Spleen	(50)	(48)	(50)
Fibrosis	5 (10%)	5 (10%)	2 (4%)
Hemosiderosis			1 (2%)
Hematopoiesis		1 (2%)	
#Ileum	(50)	(12)	(50)
Hyperplasia, lymphoid	1 (2%)		
CIRCULATORY SYSTEM			
#Lymph node	(50)	(21)	(50)
Lymphangiectasis		1 (5%)	
#Lung	(50)	(50)	(50)
Perivasculitis	5 (10%)	2 (4%)	8 (16%)
#Heart/atrium	(50)	(21)	(50)
Thrombosis, NOS	2 (4%)	1 (5%)	1 (2%)
Thrombus, mural		1 (5%)	
Degeneration, NOS			1 (2%)
Calcification, NOS		1 (5%)	
#Myocardium	(50)	(21)	(50)
Degeneration, NOS	42 (84%)	14 (67%)	45 (90%)
*Pulmonary artery	(50)	(50)	(50)
Calcification, NOS	2 (4%)		

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
DIGESTIVE SYSTEM			
#Salivary gland	(50)	(15)	(49)
Inflammation, chronic focal			1 (2%)
Atrophy, focal			1 (2%)
#Liver	(50)	(49)	(50)
Hernia, NOS			1 (2%)
Hemorrhage			1 (2%)
Inflammation, chronic focal	1 (2%)	1 (2%)	3 (6%)
Fibrosis, focal	1 (2%)		
Degeneration, cystic	6 (12%)	4 (8%)	9 (18%)
Degeneration, lipoid	3 (6%)	3 (6%)	4 (8%)
Necrosis, focal			1 (2%)
Necrosis, zonal		1 (2%)	
Basophilic cyto change	12 (24%)	20 (41%)	28 (56%)
Focal cellular change	2 (4%)		
Clear cell change	5 (10%)	7 (14%)	13 (26%)
Hyperplasia, focal	6 (12%)	3 (6%)	3 (6%)
Angiectasis	2 (4%)		
#Bile duct	(50)	(49)	(50)
Hyperplasia, NOS	44 (88%)	35 (71%)	26 (52%)
#Pancreas	(49)	(49)	(49)
Hyperplasia, focal			1 (2%)
#Pancreatic acinus	(49)	(49)	(49)
Fibrosis, focal		1 (2%)	
Degeneration, NOS			1 (2%)
Atrophy, focal	21 (43%)	17 (35%)	11 (22%)
#Stomach	(50)	(12)	(50)
Calcification, metastatic	1 (2%)		
#Gastric mucosa	(50)	(12)	(50)
Multiple cysts	7 (14%)	1 (8%)	3 (6%)
#Cardiac stomach	(50)	(12)	(50)
Inflammation, acute			1 (2%)
Inflammation, acute/chronic	1 (2%)		
Hyperplasia, epithelial		3 (25%)	3 (6%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Cyst, NOS			2 (4%)
Inflammation, chronic focal		1 (2%)	
Nephropathy	48 (96%)	35 (70%)	36 (72%)
Nephrosis, NOS	1 (2%)		
Nephrosis, cholemic			1 (2%)
Necrosis, NOS		1 (2%)	
Calcification, metastatic	1 (2%)		
#Kidney/tubule	(50)	(50)	(50)
Pigmentation, NOS	4 (8%)		6 (12%)
ENDOCRINE SYSTEM			
#Anterior pituitary	(48)	(49)	(50)
Cyst, NOS	3 (6%)	2 (4%)	3 (6%)
Hyperplasia, focal	4 (8%)	11 (22%)	9 (18%)
#Adrenal cortex	(50)	(50)	(49)
Hemorrhage	2 (4%)		
Degeneration, lipoid	15 (30%)	18 (36%)	9 (18%)
Necrosis, NOS		2 (4%)	
Hypertrophy, focal			1 (2%)
Hyperplasia, focal	7 (14%)	5 (10%)	6 (12%)
Angiectasis	2 (4%)		9 (18%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Adrenal medulla	(50)	(50)	(49)
Necrosis, NOS		1 (2%)	
Cytoplasmic vacuolization			1 (2%)
Hyperplasia, NOS			1 (2%)
Hyperplasia, focal	9 (18%)	13 (26%)	7 (14%)
#Thyroid	(50)	(14)	(50)
Inflammation, chronic focal	1 (2%)		
Hyperplasia, cystic	1 (2%)		
Hyperplasia, C-cell	6 (12%)	5 (36%)	7 (14%)
#Parathyroid	(39)	(10)	(34)
Hyperplasia, NOS	1 (3%)		
#Pancreatic islets	(49)	(49)	(49)
Hyperplasia, focal		1 (2%)	
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Hyperplasia, cystic	5 (10%)	1 (2%)	1 (2%)
*Preputial gland	(50)	(50)	(50)
Cyst, NOS	1 (2%)	1 (2%)	
Inflammation, acute/chronic	26 (52%)	38 (76%)	17 (34%)
#Prostate	(50)	(49)	(50)
Multiple cysts		1 (2%)	
Inflammation, acute/chronic	18 (36%)	25 (51%)	10 (20%)
*Seminal vesicle	(50)	(50)	(50)
Inflammation, acute			1 (2%)
Atrophy, NOS	16 (32%)	9 (18%)	13 (26%)
#Testis	(50)	(40)	(49)
Atrophy, NOS	13 (26%)	7 (18%)	8 (16%)
Hyperplasia, interstitial cell	18 (36%)	20 (50%)	25 (51%)
*Epididymis	(50)	(50)	(50)
Edema, NOS			1 (2%)
*Scrotum	(50)	(50)	(50)
Necrosis, fat		4 (8%)	
NERVOUS SYSTEM			
#Cerebrum	(50)	(13)	(50)
Hydrocephalus, NOS	1 (2%)		
Hemorrhage			2 (4%)
Hemorrhagic cyst	1 (2%)		
Degeneration, cystic			2 (4%)
Malacia			2 (4%)
#Cerebellum	(50)	(13)	(50)
Hemorrhage		1 (8%)	
Hemorrhagic cyst	1 (2%)		
SPECIAL SENSE ORGANS			
*Eye/sclera	(50)	(50)	(50)
Calcification, NOS			1 (2%)
*Eye/retina	(50)	(50)	(50)
Degeneration, NOS		6 (12%)	
*Eye/lens, cortex	(50)	(50)	(50)
Calcification, NOS		4 (8%)	
MUSCULOSKELETAL SYSTEM			
*Bone	(50)	(50)	(50)
Fibrous osteodystrophy	1 (2%)		

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
BODY CAVITIES			
*Mesentery	(50)	(50)	(50)
Necrosis, fat	3 (6%)	1 (2%)	4 (8%)
ALL OTHER SYSTEMS			
None			
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX B

SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals missing	1		
Animals necropsied	49	50	50
Animals examined histopathologically	49	50	50
INTEGUMENTARY SYSTEM			
*Subcutaneous tissue	(49)	(50)	(50)
Fibroma	1 (2%)		
Neurilemoma, malignant		1 (2%)	
RESPIRATORY SYSTEM			
#Nasal cavity	(48)	(5)	(50)
Adenoma, NOS			1 (2%)
#Lung	(49)	(13)	(50)
Adenocarcinoma, NOS, metastatic		1 (8%)	
Alveolar/bronchiolar adenoma	1 (2%)		1 (2%)
C-cell carcinoma, metastatic			1 (2%)
Pheochromocytoma, metastatic	1 (2%)		
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(50)
Leukemia, mononuclear cell	11 (22%)	14 (28%)	14 (28%)
#Spleen	(49)	(19)	(50)
Adenocarcinoma, NOS, metastatic		1 (5%)	
#Thymus	(48)	(7)	(50)
Malignant lymphoma, lymphocytic type		1 (14%)	
CIRCULATORY SYSTEM			
#Heart/atrium	(49)	(8)	(50)
Hemangiosarcoma	1 (2%)		
DIGESTIVE SYSTEM			
*Tongue	(49)	(50)	(50)
Squamous cell papilloma			1 (2%)
#Liver	(49)	(50)	(50)
Hepatocellular carcinoma	1 (2%)		
URINARY SYSTEM			
#Kidney	(49)	(6)	(50)
Tubular cell adenocarcinoma			1 (2%)
Lipoma			1 (2%)
ENDOCRINE SYSTEM			
#Pituitary	(48)	(43)	(50)
Neurilemoma, metastatic			1 (2%)
#Anterior pituitary	(48)	(43)	(50)
Adenoma, NOS	25 (52%)	24 (56%)	18 (36%)
Adenocarcinoma, NOS	1 (2%)		1 (2%)
#Adrenal cortex	(49)	(50)	(50)
Adenoma, NOS	2 (4%)	2 (4%)	
#Adrenal medulla	(49)	(50)	(50)
Pheochromocytoma	4 (8%)	2 (4%)	1 (2%)
Pheochromocytoma, malignant	1 (2%)	1 (2%)	

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Thyroid	(49)	(49)	(50)
Follicular cell adenoma	1 (2%)		1 (2%)
C-cell adenoma	7 (14%)	5 (10%)	3 (6%)
C-cell carcinoma		2 (4%)	2 (4%)
#Pancreatic islets	(49)	(6)	(50)
Islet cell adenoma	2 (4%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(49)	(50)	(50)
Adenoma, NOS	3 (6%)		
Adenocarcinoma, NOS		1 (2%)	
Papillary adenocarcinoma	1 (2%)		
Fibroadenoma	13 (27%)	16 (32%)	10 (20%)
*Clitoral gland	(49)	(50)	(50)
Adenoma, NOS	1 (2%)	2 (4%)	2 (4%)
#Uterus	(48)	(26)	(50)
Leiomyoma	1 (2%)		
Endometrial stromal polyp	13 (27%)	17 (65%)	11 (22%)
Endometrial stromal sarcoma	2 (4%)		1 (2%)
#Ovary	(49)	(8)	(50)
Granulosa cell tumor	1 (2%)		
NERVOUS SYSTEM			
#Cerebrum	(49)	(8)	(50)
Adenocarcinoma, NOS, invasive			1 (2%)
Oligodendroglioma	1 (2%)		
#Cerebellum	(49)	(8)	(50)
Medulloblastoma	1 (2%)		
*Cranial nerve	(49)	(50)	(50)
Neurilemoma, malignant			1 (2%)
SPECIAL SENSE ORGANS			
*Zymbal gland	(49)	(50)	(50)
Adenoma, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
None			
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	3	2	3
Moribund sacrifice	19	9	9
Terminal sacrifice	27	39	38
Animal missing	1		

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	46	44	41
Total primary tumors	95	88	71
Total animals with benign tumors	39	41	34
Total benign tumors	74	68	51
Total animals with malignant tumors	18	19	19
Total malignant tumors	20	20	20
Total animals with secondary tumors##	1	1	3
Total secondary tumors	1	2	3
Total animals with tumors uncertain--benign or malignant	1		
Total uncertain tumors	1		

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

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: UNTREATED CONTROL

ANIMAL NUMBER	10 06	14 02	11 07	13 00	12 07	11 03	11 04	11 06	12 04	14 04	11 05	12 03	10 07	14 06	13 07	10 02	12 02	10 08	13 09	11 00	15 00	14 00	13 06	10 01	10 03
WEEKS ON STUDY	05 51	05 53	05 55	07 44	07 75	08 22	08 33	08 44	08 85	08 55	08 88	08 88	09 00	09 00	09 93	09 94	09 94	09 96	09 96	09 98	09 98	10 00	10 02	10 04	10 04
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma	X																								
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Pheochromocytoma, metastatic												X													
Trachea	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	−	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																			X						
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	M	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS		X			X	X				X	X						X	X			X			X	X
Adenocarcinoma, NOS							X																		
Adrenal	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																					X				
Pheochromocytoma																					X	X			
Pheochromocytoma, malignant												X													
Thyroid	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									
C-cell adenoma																X								X	X
Parathyroid	+	+	−	+	−	−	+	+	M	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Pancreatic islets	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																		X							
Papillary adenocarcinoma																							X		
Fibroadenoma		X													X				X		X	X	X	X	X
Preputial/clitoral gland	N	N	N	N	N	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																			X						
Uterus	+	+	+	+	+	+	+	+	M	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																	X								
Endometrial stromal polyp				X	X									X								X			X
Endometrial stromal sarcoma					X			X																	
Ovary	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma																X									
Medulloblastoma			X																						
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell				X									X	X							X	X	X	X	X

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
•: Animals necropsied
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

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

ANIMAL NUMBER	104	105	109	111	112	118	119	120	121	125	126	128	129	131	132	133	134	135	138	141	143	145	147	148	149	TOTAL TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Fibroma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma	X																									1
Pheochromocytoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma																					X					1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	48
Adenoma, NOS		X	X		X		X		X	X			X	X	X	X	X		X	X				X	X	25
Adenocarcinoma, NOS																										1
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS																						X				2
Pheochromocytoma													X				X									4
Pheochromocytoma, malignant																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell adenoma			X																							1
C-cell adenoma				X																X		X			X	7
Parathyroid	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	40
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islet cell adenoma															X								X			2
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Adenoma, NOS										X												X				3
Papillary adenocarcinoma																										1
Fibroadenoma		X					X									X		X		X						13
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Adenoma, NOS																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leiomyoma																										1
Endometrial stromal polyp	X			X				X	X	X							X		X	X						13
Endometrial stromal sarcoma																										2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Granulosa cell tumor							X																			1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Oligodendroglioma																										1
Medulloblastoma																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Leukemia, mononuclear cell					X																X	X				11

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: LOW DOSE

ANIMAL NUMBER	04448	00899	02921	02115	01533	03355	03596	01299	01100	04505	00031	00112	00042	00045	00056	00067	00079	01111	01113	01141	01166	01177	01189	01199
WEEKS ON STUDY	03399	07909	08505	08806	08707	09902	09906	09909	10000	10000	10202	10404	10404	10404	10404	10404	10404	10404	10404	10404	10404	10404	10404	10404
INTEGUMENTARY SYSTEM																								
Subcutaneous tissue	+	+	+	+	+	N	N	N	+	+	N	N	N	N	+	N	N	N	N	N	N	N	N	N
Neurilemoma, malignant								X																
RESPIRATORY SYSTEM																								
Lungs and bronchi	+	+	+	+	+	−	−	+	−	−	+	−	−	+	+	−	−	−	−	−	−	−	−	−
Adenocarcinoma, NOS, metastatic		X																						
Trachea	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																								
Bone marrow	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Spleen	+	+	+	+	+	+	−	+	+	−	+	−	−	−	−	−	+	−	−	−	−	+	−	−
Adenocarcinoma, NOS, metastatic		X																						
Lymph nodes	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Thymus	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Malignant lymphoma, lymphocytic type																								
CIRCULATORY SYSTEM																								
Heart	+	+	+	+	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
DIGESTIVE SYSTEM																								
Salivary gland	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Esophagus	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Small intestine	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Large intestine	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−
URINARY SYSTEM																								
Kidney	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Urinary bladder	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
ENDOCRINE SYSTEM																								
Pituitary	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+
Adenoma, NOS			X		X		X			X		X						X	X	X			X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																X		X						
Pheochromocytoma																								X
Pheochromocytoma, malignant																						X		
Thyroid	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma		X																						
C-cell carcinoma																								
Parathyroid	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																								
Mammary gland	+	+	+	+	+	N	+	N	+	+	+	N	N	+	+	N	N	N	+	+	N	N	N	+
Adenocarcinoma, NOS		X																						
Fibroadenoma							X		X		X			X	X				X	X				X
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																								
Uterus	+	+	+	+	+	+	−	−	+	+	+	−	+	+	−	+	−	+	+	−	−	−	+	+
Endometrial stromal polyp						X			X	X	X		X			X		X	X					X
Ovary	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−
NERVOUS SYSTEM																								
Brain	+	+	+	+	+	−	+		−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
ALL OTHER SYSTEMS																								
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell		X				X			X		X						X					X		

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE
(Continued)

ANIMAL NUMBER	0202	0222	0233	0244	0255	0266	0277	0288	0300	0311	0322	0331	0366	0377	0388	0399	0400	0411	0422	0433	0466	0477	0488	0499	0500	TOTAL TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	N	N	N	N	N	+	N	N	+	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	*50
Neurilemoma, malignant																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	13
Adenocarcinoma, NOS, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Spleen	+	–	+	+	–	–	–	–	–	–	–	–	–	–	+	–	+	–	+	+	+	–	–	–	–	19
Adenocarcinoma, NOS, metastatic																										1
Lymph nodes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Thymus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	7
Malignant lymphoma, lymphocytic type																X										1
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	8
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
URINARY SYSTEM																										
Kidney	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
ENDOCRINE SYSTEM																										
Pituitary	+	–	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	43
Adenoma, NOS	X			X		X		X	X		X		X	X		X	X		X	X	X	X		X		24
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS																										2
Pheochromocytoma																				X						2
Pheochromocytoma, malignant																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C cell adenoma													X		X									X	X	5
C-cell carcinoma											X													X		2
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
REPRODUCTIVE SYSTEM																										
Mammary gland	N	+	+	N	N	+	N	+	+	N	+	N	+	N	N	+	N	+	N	+	N	+	N	N	N	*50
Adenocarcinoma, NOS																										1
Fibroadenoma			X			X					X		X			X		X		X		X				16
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS		X						X																		2
Uterus	–	–	–	+	+	–	+	–	+	+	+	–	–	–	–	–	–	+	+	–	–	+	–	–	+	26
Endometrial stromal polyp				X	X		X			X	X							X	X						X	17
Ovary	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, *mononuclear cell*			X	X		X									X		X		X	X	X					14

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: HIGH DOSE

ANIMAL NUMBER	070	098	088	064	069	071	083	060	072	053	054	079	051	052	055	056	057	058	059	061	062	063	065	066	067
WEEKS ON STUDY	021	061	083	086	087	088	091	094	094	099	099	099	104	104	104	104	104	104	104	104	104	104	104	104	104
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X																					
C-cell carcinoma, metastatic										X															
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS												X													
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell papilloma				X																					
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenocarcinoma																							X		
Lipoma													X												
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS			X						X	X			X				X	X	X	X		X			X
Adenocarcinoma, NOS												X													
Neurilemoma, metastatic	X																								
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma															X										
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																							X		
C-cell adenoma											X	X													
C cell carcinoma										X															
Parathyroid	−	+	+	+	+	−	+	−	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma			X									X	X						X	X			X		
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS															X										
Uterus	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp										X							X					X	X	X	X
Endometrial stromal sarcoma		X																							
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Nerves	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Neurilemoma, malignant	X																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS, invasive												X													
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N
Adenoma, NOS																X									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell			X	X		X	X	X	X					X				X				X	X		

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	0 6 8	0 7 3	0 7 4	0 7 5	0 7 6	0 7 7	0 7 8	0 8 0	0 8 1	0 8 2	0 8 4	0 8 5	0 8 6	0 8 7	0 8 9	0 9 0	0 9 1	0 9 2	0 9 3	0 9 4	0 9 5	0 9 6	0 9 7	0 9 9	1 0 0	TOTAL TISSUES TUMORS
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
C cell carcinoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	49
Nasal cavity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS																										1
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell papilloma																										1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenocarcinoma																										1
Lipoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS				X		X							X	X	X				X					X	X	18
Adenocarcinoma, NOS																										1
Neurilemoma, metastatic																										1
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma																										1
C cell adenoma									X																	3
C cell carcinoma						X																				2
Parathyroid	+	–	+	+	+	–	+	+	+	+	–	+	+	+	+	–	–	+	–	+	–	+	+	+	+	39
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroadenoma										X				X		X	X									10
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS														X												2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp	X						X					X											X			11
Endometrial stromal sarcoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Nerves	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Neurilemoma, malignant																										1
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, NOS, invasive																										1
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell							X							X				X					X			14

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Control	12,500 ppm	25,000 ppm
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	11/49 (22%)	(b,c) 14/50 (28%)	14/50 (28%)
Adjusted Rates (d)	31.2%		31.1%
Terminal Rates (e)	4/27 (15%)		8/38 (21%)
Week of First Observation	74		83
Life Table Test (f)			P=0.564N
Incidental Tumor Test (f)			P=0.187
Fisher Exact Test (f)			P=0.343
Anterior Pituitary Gland: Adenoma			
Overall Rates (a)	25/48 (52%)	24/43 (56%)	18/50 (36%)
Adjusted Rates (d)	71.9%	64.0%	43.5%
Terminal Rates (e)	17/26 (65%)	20/33 (61%)	15/38 (39%)
Week of First Observation	53	85	83
Life Table Tests (f)	P=0.003N	P=0.140N	P=0.006N
Incidental Tumor Tests (f)	P=0.028N	P=0.561N	P=0.038N
Cochran-Armitage Trend Test (f)	P=0.066N		
Fisher Exact Test (f)		P=0.442	P=0.081N
Anterior Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (a)	26/48 (54%)	24/43 (56%)	19/50 (38%)
Adjusted Rates (d)	72.5%	64.0%	44.9%
Terminal Rates (e)	17/26 (65%)	20/33 (61%)	15/38 (39%)
Week of First Observation	53	85	83
Life Table Tests (f)	P=0.004N	P=0.103N	P=0.007N
Incidental Tumor Tests (f)	P=0.034N	P=0.493N	P=0.050N
Cochran-Armitage Trend Test (f)	P=0.065N		
Fisher Exact Test (f)		P=0.521	P=0.080N
Adrenal Medulla: Pheochromocytoma			
Overall Rates (a)	4/49 (8%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (d)	13.5%	5.1%	2.6%
Terminal Rates (e)	2/27 (7%)	2/39 (5%)	1/38 (3%)
Week of First Observation	98	104	104
Life Table Tests (f)	P=0.059N	P=0.191N	P=0.102N
Incidental Tumor Tests (f)	P=0.103N	P=0.309N	P=0.181N
Cochran-Armitage Trend Test (f)	P=0.113N		
Fisher Exact Test (f)		P=0.329N	P=0.175N
Adrenal Medulla: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (a)	5/49 (10%)	3/50 (6%)	1/50 (2%)
Adjusted Rates (d)	15.6%	7.7%	2.6%
Terminal Rates (e)	2/27 (7%)	3/39 (8%)	1/38 (3%)
Week of First Observation	88	104	104
Life Table Tests (f)	P=0.031N	P=0.199N	P=0.055N
Incidental Tumor Tests (f)	P=0.065N	P=0.340N	P=0.124N
Cochran-Armitage Trend Test (f)	P=0.067N		
Fisher Exact Test (f)		P=0.346N	P=0.098N
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	7/49 (14%)	5/49 (10%)	3/50 (6%)
Adjusted Rates (d)	24.4%	12.1%	7.4%
Terminal Rates (e)	6/27 (22%)	4/39 (10%)	1/38 (3%)
Week of First Observation	94	79	99
Life Table Tests (f)	P=0.045N	P=0.177N	P=0.062N
Incidental Tumor Tests (f)	P=0.097N	P=0.244N	P=0.127N
Cochran-Armitage Trend Test (f)	P=0.115N		
Fisher Exact Test (f)		P=0.380N	P=0.151N

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Control	12,500 ppm	25,000 ppm
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	7/49 (14%)	6/49 (12%)	5/50 (10%)
Adjusted Rates (d)	24.4%	14.6%	12.2%
Terminal Rates (e)	6/27 (22%)	5/39 (13%)	2/38 (5%)
Week of First Observation	94	79	99
Life Table Tests (f)	P=0.151N	P=0.258N	P=0.182N
Incidental Tumor Tests (f)	P=0.284N	P=0.337N	P=0.349N
Cochran-Armitage Trend Test (f)	P=0.309N		
Fisher Exact Test (f)		P=0.500N	P=0.365N
Mammary Gland: Adenoma			
Overall Rates (a)	3/49 (6%)	0/50 (0%)	0/50 (0%)
Adjusted Rates (d)	10.2%	0.0%	0.0%
Terminal Rates (e)	2/27 (7%)	0/39 (0%)	0/38 (0%)
Week of First Observation	96		
Life Table Tests (f)	P=0.020N	P=0.070N	P=0.075N
Incidental Tumor Tests (f)	P=0.035N	P=0.108N	P=0.111N
Cochran-Armitage Trend Test (f)	P=0.036N		
Fisher Exact Test (f)		P=0.117N	P=0.117N
Mammary Gland: Adenoma, Adenocarcinoma, or Papillary Adenocarcinoma			
Overall Rates (a)	4/49 (8%)	1/50 (2%)	0/50 (0%)
Adjusted Rates (d)	13.4%	2.0%	0.0%
Terminal Rates (e)	2/27 (7%)	0/39 (0%)	0/38 (0%)
Week of First Observation	96	79	
Life Table Tests (f)	P=0.014N	P=0.108N	P=0.034N
Incidental Tumor Tests (f)	P=0.041N	P=0.232N	P=0.073N
Cochran-Armitage Trend Test (f)	P=0.024N		
Fisher Exact Test (f)		P=0.175N	P=0.056N
Mammary Gland: Fibroadenoma			
Overall Rates (a)	13/49 (27%)	16/50 (32%)	10/50 (20%)
Adjusted Rates (d)	38.4%	38.0%	24.6%
Terminal Rates (e)	7/27 (26%)	13/39 (33%)	8/38 (21%)
Week of First Observation	53	96	83
Life Table Tests (f)	P=0.079N	P=0.424N	P=0.110N
Incidental Tumor Tests (f)	P=0.252N	P=0.345	P=0.311N
Cochran-Armitage Trend Test (f)	P=0.264N		
Fisher Exact Test (f)		P=0.353	P=0.298N
Mammary Gland: Adenoma or Fibroadenoma			
Overall Rates (a)	16/49 (33%)	16/50 (32%)	10/50 (20%)
Adjusted Rates (d)	46.3%	38.0%	24.6%
Terminal Rates (e)	9/27 (33%)	13/39 (33%)	8/38 (21%)
Week of First Observation	53	96	83
Life Table Tests (f)	P=0.018N	P=0.184N	P=0.028N
Incidental Tumor Tests (f)	P=0.086N	P=0.554N	P=0.113N
Cochran-Armitage Trend Test (f)	P=0.098N		
Fisher Exact Test (f)		P=0.558N	P=0.115N
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	13/48 (27%)	(b) 17/26 (65%)	11/50 (22%)
Adjusted Rates (d)	40.0%		28.1%
Terminal Rates (e)	9/27 (33%)		10/38 (26%)
Week of First Observation	74		99
Life Table Test (f)			P=0.139N
Incidental Tumor Test (f)			P=0.290N
Fisher Exact Test (f)			P=0.363N

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Control	12,500 ppm	25,000 ppm
All Sites: Benign Tumors			
Overall Rates (a)	39/49 (80%)	41/50 (82%)	34/50 (68%)
Adjusted Rates (d)	90.4%	87.2%	77.2%
Terminal Rates (e)	23/27 (85%)	33/39 (85%)	28/38 (74%)
Week of First Observation	51	79	83
Life Table Tests (f)	P=0.002N	P=0.044N	P=0.005N
Incidental Tumor Tests (f)	P=0.055N	P=0.595	P=0.077N
Cochran-Armitage Trend Test (f)	P=0.106N		
Fisher Exact Test (f)		P=0.480	P=0.140N
All Sites: Malignant Tumors			
Overall Rates (a)	18/49 (37%)	19/50 (38%)	19/50 (38%)
Adjusted Rates (d)	42.2%	42.9%	39.2%
Terminal Rates (e)	4/27 (15%)	14/39 (36%)	9/38 (24%)
Week of First Observation	55	79	21
Life Table Tests (f)	P=0.297N	P=0.278N	P=0.355N
Incidental Tumor Tests (f)	P=0.150	P=0.187	P=0.110
Cochran-Armitage Trend Test (f)	P=0.490		
Fisher Exact Test (f)		P=0.531	P=0.531
All Sites: All Tumors			
Overall Rates (a)	46/49 (94%)	44/50 (88%)	41/50 (82%)
Adjusted Rates (d)	93.9%	91.7%	83.6%
Terminal Rates (e)	24/27 (89%)	35/39 (90%)	30/38 (79%)
Week of First Observation	51	79	21
Life Table Tests (f)	P=0.003N	P=0.007N	P=0.007N
Incidental Tumor Tests (f)	P=0.089N	P=0.368N	P=0.143N
Cochran-Armitage Trend Test (f)	P=0.049N		
Fisher Exact Test (f)		P=0.254N	P=0.065N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Incomplete sampling of tissues
(c) Nineteen spleens examined microscopically
(d) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(e) Observed tumor incidence at terminal kill
(f) 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 lower incidence in a dosed group is indicated by (N).

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals missing	1		
Animals necropsied	49	50	50
Animals examined histopathologically	49	50	50
INTEGUMENTARY SYSTEM			
*Skin	(49)	(50)	(50)
Epidermal inclusion cyst		2 (4%)	1 (2%)
RESPIRATORY SYSTEM			
#Nasal cavity	(48)	(5)	(50)
Inflammation, suppurative		1 (20%)	
Inflammation, acute/chronic	2 (4%)		
#Nasal turbinate	(48)	(5)	(50)
Inflammation, chronic focal	34 (71%)		26 (52%)
#Lung	(49)	(13)	(50)
Hemorrhage	1 (2%)		
Inflammation, acute	1 (2%)		
Inflammation, chronic focal		1 (8%)	2 (4%)
Alveolar macrophages	2 (4%)		
Hyperplasia, alveolar epithelium	1 (2%)		1 (2%)
Bronchiolization	1 (2%)	1 (8%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(50)
Hematopoiesis	1 (2%)		
#Bone marrow	(49)	(6)	(50)
Hypoplasia, NOS			2 (4%)
Hyperplasia, NOS	5 (10%)	2 (33%)	2 (4%)
Myelofibrosis			1 (2%)
Hyperplasia, reticulum cell	6 (12%)		3 (6%)
#Spleen	(49)	(19)	(50)
Fibrosis	1 (2%)		
Necrosis, NOS		1 (5%)	
Hemosiderosis	42 (86%)	5 (26%)	30 (60%)
Hematopoiesis	2 (4%)	2 (11%)	1 (2%)
#Ileum	(49)	(5)	(50)
Hyperplasia, lymphoid			1 (2%)
CIRCULATORY SYSTEM			
#Lung	(49)	(13)	(50)
Perivasculitis	13 (27%)	2 (15%)	10 (20%)
#Myocardium	(49)	(8)	(50)
Periarteritis			1 (2%)
Degeneration, NOS	36 (73%)	6 (75%)	41 (82%)
Necrosis, NOS			1 (2%)
Calcification, NOS			1 (2%)
*Pulmonary artery	(49)	(50)	(50)
Calcification, NOS			1 (2%)
#Pancreas	(49)	(6)	(50)
Perivasculitis	1 (2%)		
*Mesentery	(49)	(50)	(50)
Perivasculitis	1 (2%)		

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
DIGESTIVE SYSTEM			
#Salivary gland	(49)	(5)	(50)
Dilatation/ducts			1 (2%)
Necrosis, diffuse			1 (2%)
#Liver	(49)	(50)	(50)
Hernia, NOS	1 (2%)		1 (2%)
Inflammation, chronic focal	8 (16%)	7 (14%)	14 (28%)
Degeneration, lipoid	8 (16%)	3 (6%)	6 (12%)
Necrosis, focal	1 (2%)	1 (2%)	
Necrosis, zonal	1 (2%)		
Focal cellular change	40 (82%)	37 (74%)	41 (82%)
Hepatocytomegaly	3 (6%)		1 (2%)
Hyperplasia, focal		1 (2%)	
Angiectasis		1 (2%)	1 (2%)
#Bile duct	(49)	(50)	(50)
Hyperplasia, NOS	33 (67%)	24 (48%)	23 (46%)
Hyperplasia, focal	1 (2%)		
#Pancreatic acinus	(49)	(6)	(50)
Atrophy, focal	8 (16%)		10 (20%)
#Gastric mucosa	(49)	(5)	(50)
Multiple cysts	10 (20%)	3 (60%)	14 (28%)
Inflammation, acute/chronic	1 (2%)		
Calcification, NOS			1 (2%)
#Cardiac stomach	(49)	(5)	(50)
Inflammation, acute	1 (2%)		
Hyperplasia, epithelial	2 (4%)		2 (4%)
#Small intestine	(49)	(5)	(50)
Inflammation, acute/chronic		1 (20%)	
#Colon	(48)	(5)	(49)
Calcification, NOS	1 (2%)		
URINARY SYSTEM			
#Kidney	(49)	(6)	(50)
Cyst, NOS	1 (2%)	1 (17%)	
Nephropathy	35 (71%)	2 (33%)	39 (78%)
Nephrosis, NOS	2 (4%)		
Calcification, NOS			1 (2%)
#Kidney/tubule	(49)	(6)	(50)
Pigmentation, NOS	2 (4%)		5 (10%)
#Urinary bladder	(49)	(5)	(49)
Inflammation, chronic focal			2 (4%)
ENDOCRINE SYSTEM			
#Anterior pituitary	(48)	(43)	(50)
Cyst, NOS	19 (40%)	15 (35%)	23 (46%)
Hyperplasia, focal	5 (10%)	8 (19%)	15 (30%)
#Adrenal cortex	(49)	(50)	(50)
Ectopia			1 (2%)
Hemorrhage	1 (2%)		2 (4%)
Degeneration, lipoid	20 (41%)	17 (34%)	16 (32%)
Hyperplasia, focal	4 (8%)		4 (8%)
Angiectasis	21 (43%)	8 (16%)	16 (32%)
#Adrenal medulla	(49)	(50)	(50)
Calcification, focal	1 (2%)		
Hyperplasia, focal	1 (2%)	1 (2%)	
#Thyroid	(49)	(49)	(50)
Follicular cyst, NOS			1 (2%)
Hyperplasia, C-cell	18 (37%)	11 (22%)	13 (26%)
#Parathyroid	(40)	(49)	(39)
Hyperplasia, NOS			1 (3%)

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM			
*Mammary gland	(49)	(50)	(50)
Abscess, NOS			1 (2%)
Hyperplasia, cystic	15 (31%)	4 (8%)	10 (20%)
*Clitoral gland	(49)	(50)	(50)
Cyst, NOS		2 (4%)	
Inflammation, suppurative	2 (4%)	2 (4%)	3 (6%)
Abscess, NOS		1 (2%)	
Inflammation, chronic			1 (2%)
*Vagina	(49)	(50)	(50)
Prolapse		1 (2%)	
#Uterus	(48)	(26)	(50)
Dilatation, NOS			1 (2%)
Multiple cysts	3 (6%)	4 (15%)	4 (8%)
Inflammation, acute/chronic			1 (2%)
#Ovary	(49)	(8)	(50)
Cyst, NOS	1 (2%)		3 (6%)
Parovarian cyst		3 (38%)	
NERVOUS SYSTEM			
#Cerebrum	(49)	(8)	(50)
Hydrocephalus, NOS		1 (13%)	
Hemorrhagic cyst	1 (2%)		
Degeneration, cystic			1 (2%)
#Cerebellum	(49)	(8)	(50)
Hemorrhagic cyst			1 (2%)
SPECIAL SENSE ORGANS			
*Eye/retina	(49)	(50)	(50)
Degeneration, NOS		3 (6%)	3 (6%)
*Eye/crystalline lens	(49)	(50)	(50)
Degeneration, NOS			2 (4%)
*Eye/lens, capsule	(49)	(50)	(50)
Calcification, NOS		1 (2%)	1 (2%)
*Eye/lens, cortex	(49)	(50)	(50)
Calcification, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*Bone	(49)	(50)	(50)
Fibrous osteodystrophy			1 (2%)
BODY CAVITIES			
*Mesentery	(49)	(50)	(50)
Inflammation, acute/chronic			1 (2%)
Necrosis, fat	3 (6%)	3 (6%)	6 (12%)
ALL OTHER SYSTEMS			
None			
SPECIAL MORPHOLOGY SUMMARY			
Animal missing/no necropsy	1		

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX C

SUMMARY OF LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals missing			1
Animals necropsied	49	50	48
Animals examined histopathologically	49	49	48
INTEGUMENTARY SYSTEM			
*Subcutaneous tissue	(49)	(50)	(48)
Sarcoma, NOS			2 (4%)
Fibroma	1 (2%)	1 (2%)	1 (2%)
Fibrosarcoma	2 (4%)	3 (6%)	3 (6%)
RESPIRATORY SYSTEM			
#Lung	(49)	(15)	(48)
Hepatocellular carcinoma, metastatic	2 (4%)		
Alveolar/bronchiolar adenoma	6 (12%)	3 (20%)	4 (8%)
Alveolar/bronchiolar carcinoma		3 (20%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(48)
Malignant lymphoma, NOS	1 (2%)		
Malignant lymphoma, undifferentiated type	1 (2%)	2 (4%)	
Malignant lymphoma, lymphocytic type	1 (2%)		
Malignant lymphoma, histiocytic type	2 (4%)		
Malignant lymphoma, mixed type	3 (6%)	1 (2%)	1 (2%)
#Spleen	(48)	(49)	(47)
Malignant lymphoma, mixed type			1 (2%)
#Mesenteric lymph node	(49)	(48)	(48)
Malignant lymphoma, mixed type	1 (2%)		
*Intestinal tract	(49)	(50)	(48)
Malignant lymphoma, undifferentiated type	1 (2%)		
CIRCULATORY SYSTEM			
#Spleen	(48)	(49)	(47)
Hemangiosarcoma		1 (2%)	
#Liver	(49)	(16)	(48)
Hemangiosarcoma	1 (2%)	1 (6%)	1 (2%)
DIGESTIVE SYSTEM			
#Liver	(49)	(16)	(48)
Hepatocellular adenoma	7 (14%)	8 (50%)	7 (15%)
Hepatocellular carcinoma	5 (10%)	4 (25%)	3 (6%)
URINARY SYSTEM			
#Kidney	(49)	(5)	(48)
Tubular cell adenocarcinoma	1 (2%)		
ENDOCRINE SYSTEM			
#Thyroid	(49)	(3)	(47)
Follicular cell adenoma	1 (2%)		
REPRODUCTIVE SYSTEM			
None			

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(49)	(50)	(48)
Adenoma, NOS	4 (8%)		1 (2%)
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(50)	(48)
Fibrosarcoma			1 (2%)
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	11	4	4
Moribund sacrifice	8	3	2
Terminal sacrifice	31	43	43
Animal missing			1
TUMOR SUMMARY			
Total animals with primary tumors**	31	23	21
Total primary tumors	38	27	25
Total animals with benign tumors	16	12	12
Total benign tumors	19	12	13
Total animals with malignant tumors	17	14	11
Total malignant tumors	19	15	12
Total animals with secondary tumors##	2		
Total secondary tumors	2		

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

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: UNTREATED CONTROL

ANIMAL NUMBER	11 17	12 29	13 35	12 23	10 05	11 11	10 06	11 18	11 16	11 19	13 32	10 01	14 47	14 40	11 15	12 27	10 02	11 10	13 36	10 03	10 04	10 07	10 08	10 09	11 12
WEEKS ON STUDY	01 13	02 28	05 99	06 99	07 74	08 81	08 82	08 83	08 84	08 84	08 85	08 89	08 89	09 97	09 98	09 98	10 01	10 03	10 33	10 44	10 44	10 44	10 44	10 44	10 44
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	A	+	N	+	+	N	+	+	+	+	+	+	+	+
Fibroma																									
Fibrosarcoma								X											X						
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic															X						X				
Alveolar/bronchiolar adenoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																									
Thymus	+	+	+	+	+	A	+	+	–	+	+	A	+	+	+	+	+	+	+	+	–	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma									X																
Hepatocellular carcinoma											X		X		X						X				
Hemangiosarcoma														X											
Bile duct	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	N	+	N	+	+	+	N	+	+	N	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	–	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	–	–	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	–	–	–	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	–	+	+	–	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenocarcinoma																									
Urinary bladder	–	–	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									
Parathyroid	–	+	+	–	+	+	+	+	+	+	–	A	–	+	+	+	–	+	–	–	–	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	–	+	+	–	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	–	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS																X									
Malignant lymphoma, undifferentiated type																									
Malignant lymphoma, lymphocytic type	X																								
Malignant lymphoma, histiocytic type				X													X								
Malignant lymphoma, mixed type																									
Intestinal tract																									
Malignant lymphoma, undifferentiated type												A													

+: Tissue examined microscopically
–: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
* Animals necropsied

: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

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

ANIMAL NUMBER	113	114	120	121	122	124	125	126	128	130	131	133	134	137	138	139	141	142	143	144	145	146	148	149	150	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Fibroma										X																1
Fibrosarcoma																										2
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma, metastatic																										2
Alveolar/bronchiolar adenoma			X				X					X				X						X		X		6
Trachea	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Malignant lymphoma, mixed type								X																		1
Thymus	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+		44
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma														X	X			X				X	X			7
Hepatocellular carcinoma									X																	5
Hemangiosarcoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	*49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tubular cell adenocarcinoma								X																		1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell adenoma										X																1
Parathyroid	+	+	−	−	−	−	+	−	+	−	−	+	+	+	−	+	+	+	+	−	+	+	+	+	+	32
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	46
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Adenoma, NOS	X																X						X		X	4
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Malignant lymphoma, NOS																										1
Malig. lymphoma, undifferentiated type																				X						1
Malignant lymphoma, lymphocytic type																										1
Malignant lymphoma, histiocytic type																										2
Malignant lymphoma, mixed type					X									X			X									3
Intestinal tract																										
Malig. lymphoma, undifferentiated type														X												1

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: LOW DOSE

ANIMAL NUMBER	01 22	02 21	01 11	00 08	00 04	01 44	04 55	00 01	00 02	00 03	00 05	00 06	00 07	00 09	01 10	01 13	01 15	01 16	01 17	01 18	01 19	02 20	02 21	02 23	02 24
WEEKS ON STUDY	02 24	08 44	08 89	09 97	09 99	10 01	10 01	10 04	10 04	10 44	10 04	10 44	10 04	10 44	10 04	10 04	10 04	10 04	10 04	10 04	10 04	10 04	*0 44	10 04	10 04
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N
Fibroma																									
Fibrosarcoma							X				X														
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	A	–	–	+	–	–	+	–	+	+	–	+	+	+	–	–	–	..	–	–	–
Alveolar/bronchiolar adenoma																	X	X							
Alveolar/bronchiolar carcinoma				X				X					X												
Trachea	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spleen	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																			X						
Lymph nodes	+	+	+	+	A	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Liver	+	+	+	–	A	–	–	+	–	–	–	–	–	–	–	+	–	+	–	+	–	–	+	+	+
Hepatocellular adenoma		X						X								X				X					X
Hepatocellular carcinoma																		X					X	X	
Hemangiosarcoma																									
Bile duct	+	+	+	–	A	–	–	+	–	–	–	–	–	–	–	+	–	+	–	+	–	–	+	+	+
Gallbladder & common bile duct	+	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	–	A	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Esophagus	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Stomach	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Small intestine	–	+	+	–	A	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Large intestine	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
URINARY SYSTEM																									
Kidney	+	+	+	+	A	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Urinary bladder	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Adrenal	+	+	+	+	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Thyroid	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Parathyroid	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Prostate	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NERVOUS SYSTEM																									
Brain	+	+	+	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, undifferentiated type								X																	
Malignant lymphoma, mixed type																									

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	025	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	046	047	048	049	050	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Fibroma																	X									1
Fibrosarcoma														X												3
RESPIRATORY SYSTEM																										
Lungs and bronchi	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	15
Alveolar/bronchiolar adenoma						X																				3
Alveolar/bronchiolar carcinoma																										3
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
Liver	+	–	–	–	–	–	–	+	–	–	+	+	–	–	–	–	–	–	–	+	+	–	–	–	–	16
Hepatocellular adenoma	X							X												X						8
Hepatocellular carcinoma											X															4
Hemangiosarcoma	X																									1
Bile duct	+	–	–	–	–	–	–	+	–	–	+	+	–	–	–	–	–	–	–	+	+	–	–	–	–	16
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Pancreas	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	4
URINARY SYSTEM																										
Kidney	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
ENDOCRINE SYSTEM																										
Pituitary	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
Adrenal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
Thyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
Parathyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
Prostate	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malig. lymphoma, undifferentiated type												X														2
Malignant lymphoma, mixed type																								X		1

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: HIGH DOSE

ANIMAL NUMBER	089	051	061	060	067	071	094	052	053	054	055	056	057	058	059	062	063	064	065	066	068	069	070	072	073
WEEKS ON STUDY	061	084	087	089	094	095	101	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS							X																		
Fibroma																									
Fibrosarcoma																		X							
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X				X			X										
Trachea	+	+	+	A	+	M	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	–	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																		X							
Lymph nodes	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	–	+	A	+	M	–	+	+	+	+	+	–	–	–	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Liver	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma									X							X							X		
Hepatocellular carcinoma								X																	
Hemangiosarcoma														X											
Bile duct	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	A	+	M	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+
Pancreas	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Stomach	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	–	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	A	+	M	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Adrenal	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Thyroid	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Parathyroid	+	+	–	A	+	M	+	+	–	+	+	–	+	+	+	+	–	–	+	–	+	–	–	+	–
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	N	A	N	M	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	A	+	M	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	A	+	M	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	A	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS													X												
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	A	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrosarcoma		X																							
Malignant lymphoma, mixed type																	X								

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	074	075	076	077	078	079	080	081	082	083	084	085	086	087	088	090	091	092	093	095	096	097	098	099	100	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*48
Sarcoma, NOS																		X								2
Fibroma	X																									1
Fibrosarcoma																	X		X							3
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma													X													4
Trachea	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Malignant lymphoma, mixed type																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+	40
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular adenoma	X		X		X												X									7
Hepatocellular carcinoma												X													X	3
Hemangiosarcoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*48
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	46
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Parathyroid	–	+	+	+	+	–	–	+	+	–	–	+	–	–	–	–	+	+	+	+	+	+	+	–	+	29
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*48
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*48
Adenoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*48
Fibrosarcoma																										1
Malignant lymphoma, mixed type																										1

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Control	12,500 ppm	25,000 ppm
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	2/49 (4%)	3/50 (6%)	3/48 (6%)
Adjusted Rates (b)	5.3%	6.8%	7.0%
Terminal Rates (c)	0/31 (0%)	2/43 (5%)	3/43 (7%)
Week of First Observation	83	101	104
Life Table Tests (d)	P=0.540	P=0.618	P=0.622
Incidental Tumor Tests (d)	P=0.303	P=0.381	P=0.392
Cochran-Armitage Trend Test (d)	P=0.402		
Fisher Exact Test (d)		P=0.510	P=0.490
Subcutaneous Tissue: Sarcoma or Fibrosarcoma			
Overall Rates (a)	2/49 (4%)	3/50 (6%)	5/48 (10%)
Adjusted Rates (b)	5.3%	6.8%	11.4%
Terminal Rates (c)	0/31 (0%)	2/43 (5%)	4/43 (9%)
Week of First Observation	83	101	101
Life Table Tests (d)	P=0.259	P=0.618	P=0.346
Incidental Tumor Tests (d)	P=0.078	P=0.381	P=0.112
Cochran-Armitage Trend Test (d)	P=0.150		
Fisher Exact Test (d)		P=0.510	P=0.209
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	3/49 (6%)	4/50 (8%)	4/48 (8%)
Adjusted Rates (b)	8.3%	9.0%	9.3%
Terminal Rates (c)	1/31 (3%)	3/43 (7%)	4/43 (9%)
Week of First Observation	83	101	104
Life Table Tests (d)	P=0.573N	P=0.648	P=0.651N
Incidental Tumor Tests (d)	P=0.375	P=0.451	P=0.460
Cochran-Armitage Trend Test (d)	P=0.412		
Fisher Exact Test (d)		P=0.511	P=0.488
Subcutaneous Tissue: Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates (a)	3/49 (6%)	4/50 (8%)	6/48 (13%)
Adjusted Rates (b)	8.3%	9.0%	13.6%
Terminal Rates (c)	1/31 (3%)	3/43 (7%)	5/43 (12%)
Week of First Observation	83	101	101
Life Table Tests (d)	P=0.322	P=0.648	P=0.404
Incidental Tumor Tests (d)	P=0.129	P=0.451	P=0.172
Cochran-Armitage Trend Test (d)	P=0.177		
Fisher Exact Test (d)		P=0.511	P=0.233
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	6/49 (12%)	(e, f) 6/15 (40%)	4/48 (8%)
Adjusted Rates (b)	19.4%		9.3%
Terminal Rates (c)	6/31 (19%)		4/43 (9%)
Week of First Observation	104		104
Life Table Test (d)			P=0.185N
Incidental Tumor Test (d)			P=0.185N
Fisher Exact Test (d)			P=0.384N
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	4/49 (8%)	(e,g) 1/50 (2%)	2/48 (4%)
Adjusted Rates (b)	12.9%		4.7%
Terminal Rates (c)	4/31 (13%)		2/43 (5%)
Week of First Observation	104		104
Life Table Test (d)			P=0.199N
Incidental Tumor Test (d)			P=0.199N
Fisher Exact Test (d)			P=0.349N

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Control	12,500 ppm	25,000 ppm
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	9/49 (18%)	(e,g) 3/50 (6%)	2/48 (4%)
Adjusted Rates (b)	24.1%		4.7%
Terminal Rates (c)	5/31 (16%)		2/43 (5%)
Week of First Observation	13		104
Life Table Test (d)			P=0.010N
Incidental Tumor Test (d)			P=0.054N
Fisher Exact Test (d)			P=0.028N
Liver: Hepatocellular Adenoma			
Overall Rates (a)	7/49 (14%)	(e) 8/16 (50%)	7/48 (15%)
Adjusted Rates (b)	20.6%		16.3%
Terminal Rates (c)	5/31 (16%)		7/43 (16%)
Week of First Observation	84		104
Life Table Test (d)			P=0.369N
Incidental Tumor Test (d)			P=0.513N
Fisher Exact Test (d)			P=0.597
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	5/49 (10%)	(e) 4/16 (25%)	3/48 (6%)
Adjusted Rates (b)	13.7%		7.0%
Terminal Rates (c)	2/31 (6%)		3/43 (7%)
Week of First Observation	85		104
Life Table Test (d)			P=0.223N
Incidental Tumor Test (d)			P=0.469N
Fisher Exact Test (d)			P=0.369N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	12/49 (24%)	(e) 12/16 (75%)	10/48 (21%)
Adjusted Rates (b)	32.4%		23.3%
Terminal Rates (c)	7/31 (23%)		10/43 (23%)
Week of First Observation	84		104
Life Table Test (d)			P=0.157N
Incidental Tumor Test (d)			P=0.392N
Fisher Exact Test (d)			P=0.426N
Harderian Gland: Adenoma			
Overall Rates (a)	4/49 (8%)	0/50 (0%)	1/48 (2%)
Adjusted Rates (b)	12.9%	0.0%	2.3%
Terminal Rates (c)	4/31 (13%)	0/43 (0%)	1/43 (2%)
Week of First Observation	104		104
Life Table Tests (d)	P=0.041N	P=0.030N	P=0.095N
Incidental Tumor Tests (d)	P=0.041N	P=0.030N	P=0.095N
Cochran-Armitage Trend Test (d)	P=0.084N		
Fisher Exact Test (d)		P=0.056N	P=0.187N
All Sites: Benign Tumors			
Overall Rates (a)	16/49 (33%)	12/50 (24%)	12/48 (25%)
Adjusted Rates (b)	48.1%	27.1%	27.9%
Terminal Rates (c)	14/31 (45%)	11/43 (26%)	12/43 (28%)
Week of First Observation	84	84	104
Life Table Tests (d)	P=0.038N	P=0.047N	P=0.043N
Incidental Tumor Tests (d)	P=0.074N	P=0.091N	P=0.074N
Cochran-Armitage Trend Test (d)	P=0.231N		
Fisher Exact Test (d)		P=0.232N	P=0.272N

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Control	12,500 ppm	25,000 ppm
All Sites: Malignant Tumors			
Overall Rates (a)	17/49 (35%)	14/50 (28%)	11/48 (23%)
Adjusted Rates (b)	40.4%	31.0%	24.3%
Terminal Rates (c)	7/31 (23%)	12/43 (28%)	9/43 (21%)
Week of First Observation	13	97	84
Life Table Tests (d)	P=0.026N	P=0.108N	P=0.040N
Incidental Tumor Tests (d)	P=0.259N	P=0.437N	P=0.414N
Cochran-Armitage Trend Test (d)	P=0.121N		
Fisher Exact Test (d)		P=0.308N	P=0.146N
All Sites: All Tumors			
Overall Rates (a)	31/49 (63%)	23/50 (46%)	21/48 (44%)
Adjusted Rates (b)	71.8%	49.9%	46.6%
Terminal Rates (c)	19/31 (61%)	20/43 (47%)	19/43 (44%)
Week of First Observation	13	84	84
Life Table Tests (d)	P=0.001N	P=0.005N	P=0.002N
Incidental Tumor Tests (d)	P=0.036N	P=0.054N	P=0.056N
Cochran-Armitage Trend Test (d)	P=0.034N		
Fisher Exact Test (d)		P=0.064N	P=0.042N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(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 lower incidence in a dosed group is indicated by (N).
(e) Incomplete sampling of tissues
(f) Includes three carcinomas; no other carcinomas were observed in any dose group.
(g) Sixteen livers examined microscopically

TABLE C4. HISTORICAL INCIDENCE OF HEMATOPOIETIC SYSTEM TUMORS IN MALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls	
	Lymphoma	Lymphoma or Leukemia
Historical Incidence at Physiological Research Laboratories		
Ephedrine sulfate	8/50	8/50
Phenylephrine hydrochloride	3/50	3/50
Oxytetracycline hydrochloride	8/50	8/50
TOTAL	19/150 (12.7%)	19/150 (12.7%)
SD (b)	5.77%	5.77%
Range (c)		
High	8/50	8/50
Low	3/50	3/50
Overall Historical Incidence		
TOTAL	239/2,040 (11.7%)	243/2,040 (11.9%)
SD (b)	7.01%	7.13%
Range (c)		
High	16/50	16/50
Low	1/50	1/50

(a) Data as of August 7, 1986, for studies of at least 104 weeks
(b) Standard deviation
(c) 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 FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals missing			1
Animals necropsied	49	50	48
Animals examined microscopically	49	49	48
INTEGUMENTARY SYSTEM			
*Skin	(49)	(50)	(48)
Inflammation, acute focal			1 (2%)
Inflammation, acute/chronic	1 (2%)		1 (2%)
Inflammation, chronic	1 (2%)	1 (2%)	
Inflammation, chronic focal	2 (4%)		1 (2%)
Inflammation, chronic necrotizing			1 (2%)
Inflammation with fibrosis		2 (4%)	
*Subcutaneous tissue	(49)	(50)	(48)
Abscess, NOS	1 (2%)		
Inflammation, acute/chronic	1 (2%)		
Inflammation, chronic suppurative			1 (2%)
Inflammation with fibrosis	1 (2%)	1 (2%)	
RESPIRATORY SYSTEM			
#Lung	(49)	(15)	(48)
Congestion, NOS		2 (13%)	
Hemorrhage	1 (2%)		1 (2%)
Pneumonia, interstitial chronic	1 (2%)	3 (20%)	6 (13%)
Alveolar macrophages		1 (7%)	1 (2%)
Hyperplasia, alveolar epithelium	1 (2%)		3 (6%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(48)
Hyperplasia, lymphoid			1 (2%)
#Bone marrow	(49)	(3)	(48)
Fibrosis, multifocal	1 (2%)		
Hyperplasia, granulocytic	4 (8%)	1 (33%)	3 (6%)
#Spleen	(48)	(49)	(47)
Angiectasis	2 (4%)		1 (2%)
Hyperplasia, reticulum cell			2 (4%)
Hyperplasia, lymphoid	5 (10%)	8 (16%)	6 (13%)
Hematopoiesis	7 (15%)	4 (8%)	6 (13%)
#Lymph node	(49)	(48)	(48)
Inflammation, chronic			1 (2%)
Plasma cell infiltrate	1 (2%)		
#Mandibular lymph node	(49)	(48)	(48)
Hyperplasia, lymphoid	1 (2%)		4 (8%)
#Mesenteric lymph node	(49)	(48)	(48)
Hemorrhage		1 (2%)	
Inflammation, chronic		1 (2%)	1 (2%)
Hyperplasia, NOS	2 (4%)	2 (4%)	2 (4%)
Angiectasis	4 (8%)		
Hyperplasia, lymphoid	1 (2%)	2 (4%)	
#Salivary gland	(48)	(3)	(46)
Hyperplasia, lymphoid	6 (13%)		8 (17%)
#Liver	(49)	(16)	(48)
Hyperplasia, lymphoid	1 (2%)		1 (2%)
#Glandular stomach	(47)	(3)	(48)
Hyperplasia, lymphoid	1 (2%)		
#Cecum	(47)	(4)	(48)
Hyperplasia, lymphoid	1 (2%)		3 (6%)
#Kidney	(49)	(5)	(48)
Hyperplasia, lymphoid	8 (16%)		10 (21%)

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Kidney/pelvis	(49)	(5)	(48)
Hyperplasia, lymphoid			1 (2%)
#Thymus	(44)	(49)	(40)
Cyst, NOS	1 (2%)	1 (2%)	
Hyperplasia, lymphoid			3 (8%)
#Thymic cortex	(44)	(49)	(40)
Necrosis, NOS		1 (2%)	
#Thymic medulla	(44)	(49)	(40)
Inflammation, suppurative		1 (2%)	
CIRCULATORY SYSTEM			
#Lung	(49)	(15)	(48)
Thrombosis, NOS			1 (2%)
#Heart	(49)	(3)	(48)
Inflammation, chronic necrotizing			1 (2%)
Fibrosis, focal	1 (2%)		
#Heart/atrium	(49)	(3)	(48)
Thrombus, mural		1 (33%)	
#Myocardium/left ventricle	(49)	(3)	(48)
Perivasculitis			1 (2%)
#Liver	(49)	(16)	(48)
Thrombus, organized			1 (2%)
DIGESTIVE SYSTEM			
#Salivary gland	(48)	(3)	(46)
Inflammation, chronic focal			1 (2%)
#Liver	(49)	(16)	(48)
Multiple cysts		1 (6%)	
Inflammation, acute	2 (4%)		
Inflammation, acute necrotizing			1 (2%)
Inflammation, chronic		1 (6%)	
Necrosis, NOS	2 (4%)	1 (6%)	2 (4%)
Infarct, NOS	1 (2%)		
Focal cellular change	1 (2%)		1 (2%)
Angiectasis		1 (6%)	
*Gallbladder	(49)	(50)	(48)
Distention			1 (2%)
Hyperplasia, epithelial	1 (2%)		
#Gastric mucosa	(47)	(3)	(48)
Abscess, NOS			1 (2%)
#Glandular stomach	(47)	(3)	(48)
Cyst, NOS			1 (2%)
Inflammation, acute/chronic			1 (2%)
Eosinophilic leukocytic infiltrate			3 (6%)
Inflammation, chronic			2 (4%)
#Cecum	(47)	(4)	(48)
Dilatation, NOS		1 (25%)	
*Anus	(49)	(50)	(48)
Inflammation, acute/chronic			1 (2%)

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM			
#Kidney	(49)	(5)	(48)
Cyst, NOS	1 (2%)		
Hemorrhage	1 (2%)		
Glomerulonephritis, NOS	2 (4%)	1 (20%)	
Pyelonephritis, acute	2 (4%)		
Glomerulonephritis, chronic			2 (4%)
Inflammation, chronic focal	1 (2%)		
Necrosis, coagulative		1 (20%)	
Calcification, focal	1 (2%)		
#Kidney/tubule	(49)	(5)	(48)
Dilatation, NOS		1 (20%)	1 (2%)
Multiple cysts	1 (2%)		
Degeneration, hyaline	1 (2%)		
#Urinary bladder	(47)	(3)	(48)
Hemorrhage			1 (2%)
Inflammation, acute	1 (2%)		1 (2%)
Inflammation, acute/chronic	1 (2%)		
Necrosis, NOS			1 (2%)
*Urethra	(49)	(50)	(48)
Inflammation, acute suppurative	1 (2%)		
ENDOCRINE SYSTEM			
#Anterior pituitary	(49)	(3)	(47)
Cyst, NOS	5 (10%)		2 (4%)
Multiple cysts	1 (2%)		1 (2%)
Hyperplasia, focal	3 (6%)		1 (2%)
#Adrenal/capsule	(49)	(4)	(47)
Hyperplasia, stromal	33 (67%)	3 (75%)	34 (72%)
#Adrenal cortex	(49)	(4)	(47)
Degeneration, lipoid	1 (2%)		
Hypertrophy, focal	2 (4%)		
Hyperplasia, focal			1 (2%)
#Thyroid	(49)	(3)	(47)
Cyst, NOS			1 (2%)
Cystic follicles	2 (4%)		5 (11%)
REPRODUCTIVE SYSTEM			
*Penis	(49)	(50)	(48)
Inflammation, chronic		1 (2%)	
*Prepuce	(49)	(50)	(48)
Inflammation, chronic	1 (2%)		1 (2%)
Inflammation, chronic focal	1 (2%)		
Inflammation, chronic suppurative		1 (2%)	1 (2%)
Melanin	1 (2%)		
*Preputial gland	(49)	(50)	(48)
Cystic ducts	6 (12%)	5 (10%)	
Inflammation, suppurative			1 (2%)
Inflammation, acute	1 (2%)		
Inflammation, acute/chronic	3 (6%)		1 (2%)
Inflammation, chronic	4 (8%)	6 (12%)	
Inflammation, chronic suppurative	2 (4%)	6 (12%)	1 (2%)
Abscess, chronic	1 (2%)		
Hyperplasia, cystic	2 (4%)		3 (6%)
#Prostate	(48)	(4)	(47)
Inflammation, acute	1 (2%)		1 (2%)
Inflammation, chronic	1 (2%)		
Necrosis, NOS	1 (2%)		
Hyperplasia, cystic	1 (2%)		1 (2%)

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM (Continued)			
*Seminal vesicle	(49)	(50)	(48)
Distention	15 (31%)	20 (40%)	8 (17%)
Inflammation, acute			1 (2%)
Inflammation, acute/chronic		1 (2%)	
Inflammation, chronic suppurative	1 (2%)		
Hyperplasia, cystic	3 (6%)		
#Testis	(46)	(4)	(47)
Calcification, focal	1 (2%)	1 (25%)	5 (11%)
NERVOUS SYSTEM			
#Brain	(49)	(3)	(48)
Demyelinization			1 (2%)
Calcification, focal	23 (47%)	2 (67%)	19 (40%)
SPECIAL SENSE ORGANS			
*Ear canal	(49)	(50)	(48)
Inflammation, acute/chronic	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Joint of lower extremities	(49)	(50)	(48)
Ankylosis	12 (24%)	11 (22%)	9 (19%)
BODY CAVITIES			
*Mesentery	(49)	(50)	(48)
Inflammation, chronic focal	1 (2%)		
Necrosis, coagulative		1 (2%)	
ALL OTHER SYSTEMS			
Adipose tissue			
Necrosis, fat	1	1	6
Calcification, focal	1		1
SPECIAL MORPHOLOGY SUMMARY			
No lesion reported		1	
Animal missing/no necropsy			1
Auto/necropsy/histo perf	1		
Auto/necropsy/no histo		1	
Autolysis/no necropsy	1		1

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX D

SUMMARY OF LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals missing	1	1	
Animals necropsied	49	48	50
Animals examined histopathologically	49	48	50
INTEGUMENTARY SYSTEM			
*Skin	(49)	(48)	(50)
Papilloma, NOS			1 (2%)
*Subcutaneous tissue	(49)	(48)	(50)
Fibrosarcoma	2 (4%)	1 (2%)	
RESPIRATORY SYSTEM			
#Lung	(49)	(48)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	1 (2%)	5 (10%)
Alveolar/bronchiolar carcinoma	2 (4%)		2 (4%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(48)	(50)
Malignant lymphoma, NOS	1 (2%)	1 (2%)	5 (10%)
Malignant lymphoma, undifferentiated type	2 (4%)	2 (4%)	2 (4%)
Malignant lymphoma, lymphocytic type			3 (6%)
Malignant lymphoma, histiocytic type		1 (2%)	
Malignant lymphoma, mixed type	7 (14%)	4 (8%)	3 (6%)
#Spleen	(49)	(45)	(49)
Malignant lymphoma, NOS	1 (2%)	1 (2%)	1 (2%)
Malignant lymphoma, mixed type	2 (4%)	1 (2%)	
#Lymph node	(48)	(13)	(49)
Malignant lymphoma, lymphocytic type	1 (2%)		
#Mesenteric lymph node	(48)	(13)	(49)
Malignant lymphoma, mixed type	1 (2%)		
#Small intestine	(49)	(5)	(50)
Malignant lymphoma, mixed type	1 (2%)		
CIRCULATORY SYSTEM			
*Neck	(49)	(48)	(50)
Hemangiosarcoma			1 (2%)
*Lumbar region	(49)	(48)	(50)
Hemangiosarcoma			1 (2%)
#Spleen	(49)	(45)	(49)
Hemangiosarcoma			1 (2%)
#Liver	(49)	(48)	(50)
Hemangiosarcoma		1 (2%)	
DIGESTIVE SYSTEM			
*Tongue	(49)	(48)	(50)
Papilloma, NOS	1 (2%)		
#Liver	(49)	(48)	(50)
Hepatocellular adenoma	8 (16%)		
Hepatocellular carcinoma	3 (6%)		
URINARY SYSTEM			
None			

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
#Anterior pituitary	(48)	(46)	(49)
Carcinoma, NOS	1 (2%)		
Adenoma, NOS	11 (23%)	2 (4%)	5 (10%)
#Adrenal medulla	(49)	(5)	(50)
Pheochromocytoma	1 (2%)		1 (2%)
#Thyroid	(49)	(47)	(50)
Follicular cell adenoma	4 (8%)		
#Pancreatic islets	(49)	(6)	(49)
Islet cell adenoma			1 (2%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(49)	(48)	(50)
Adenocarcinoma, NOS	1 (2%)		
#Uterus	(49)	(44)	(50)
Leiomyosarcoma		1 (2%)	
Endometrial stromal polyp		1 (2%)	
#Cervix uteri	(49)	(44)	(50)
Leiomyosarcoma			1 (2%)
#Ovary	(48)	(16)	(50)
Papillary adenoma		1 (6%)	
Granulosa cell tumor	1 (2%)	1 (6%)	
Teratoma, benign			1 (2%)
Choriocarcinoma		1 (6%)	
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(49)	(48)	(50)
Adenoma, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(48)	(50)
Alveolar/bronchiolar carcinoma, metastatic	1 (2%)		
Osteosarcoma		1 (2%)	
Osteosarcoma, metastatic	1 (2%)	1 (2%)	
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	5	9	4
Moribund sacrifice	7	5	8
Terminal sacrifice	37	34	38
Accidentally killed, nda		1	
Animal missing	1	1	

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	34	18	26
Total primary tumors	54	21	34
Total animals with benign tumors	19	5	14
Total benign tumors	28	5	14
Total animals with malignant tumors	23	14	19
Total malignant tumors	25	15	20
Total animals with secondary tumors##	2	1	
Total secondary tumors	2	1	
Total animals with tumors uncertain--benign or malignant	1	1	
Total uncertain tumors	1	1	

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

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: UNTREATED CONTROL

ANIMAL NUMBER	13 8	10 3	14 6	14 8	13 9	11 3	12 2	11 2	12 4	12 5	12 7	12 0	14 5	10 1	10 2	10 4	10 5	10 6	10 7	10 8	10 9	11 0	11 1	11 4	11 5
WEEKS ON STUDY	05 7	06 9	08 4	08 4	09 5	09 9	09 9	10 0	10 2	10 2	10 2	10 3	10 3	10 4	10 4	10 4	10 4	10 4	10 4	10 4	10 4	10 4	10 4	10 4	10 4
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma					X																		X		
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma										X														X	
Alveolar/bronchiolar carcinoma							X																		
Trachea	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, NOS															X										
Malignant lymphoma, mixed type																									
Lymph nodes	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, lymphocytic type			X																						
Malignant lymphoma, mixed type																									
Thymus	+	M	−	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	M	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Papilloma, NOS													X												
Salivary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma							X																	X	
Hepatocellular carcinoma							X					X													
Bile duct	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																					X				
Large intestine	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	M	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS												X													
Adenoma, NOS													X		X	X		X		X					
Adrenal	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									
Thyroid	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma									X				X												
Parathyroid	+	M	+	A	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Uterus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	−	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor																X									
NERVOUS SYSTEM																									
Brain	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																								X	
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Alveolar/bronchiolar carcinoma, metastatic																									
Osteosarcoma, metastatic	X																								
Malignant lymphoma, NOS						X																			
Malignant lymphoma, undifferentiated type																		X							
Malignant lymphoma, mixed type													X				X		X	X		X			

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
* Animals necropsied

: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

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

ANIMAL NUMBER	116	117	118	119	121	123	126	128	129	130	131	132	133	134	135	136	137	140	141	142	143	144	147	149	150	TOTAL TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Fibrosarcoma																										2
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma																										2
Alveolar/bronchiolar carcinoma											X															2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Malignant lymphoma, NOS																										1
Malignant lymphoma, mixed type					X																	X				2
Lymph nodes	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Malignant lymphoma, lymphocytic type																										1
Malignant lymphoma, mixed type																				X						1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	+	–	+	–	41
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Papilloma, NOS																										1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma	X	X	X	X								X						X								8
Hepatocellular carcinoma																			X							3
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Malignant lymphoma, mixed type																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, NOS																										1
Adenoma, NOS			X	X								X						X	X	X						11
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma																									X	1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell adenoma											X		X													4
Parathyroid	–	+	+	+	+	+	+	+	–	+	+	–	+	–	+	–	+	–	+	+	–	–	+	+	–	36
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Adenocarcinoma, NOS															X											1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Granulosa cell tumor																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Adenoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Alveolar/bronchiolar carcinoma, metast											X															1
Osteosarcoma, metastatic																										1
Malignant lymphoma, NOS																										1
Malig lymphoma, undifferentiated type																							X			2
Malignant lymphoma, mixed type						X																		X		7

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: LOW DOSE

ANIMAL NUMBER	0021	0028	0002	0050	0031	0042	0024	0014	0008	0022	0046	0036	0006	0045	0049	0001	0003	0004	0005	0007	0009	0010	0011	0012	0013
WEEKS ON STUDY	035	039	048	055	071	084	089	093	097	100	100	101	103	103	103	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	M	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrosarcoma									X																
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																					X				
Trachea	+	+	+	+	−	M	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
HEMATOPOIETIC SYSTEM																									
Bone marrow	−	+	+	+	+	M	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
Spleen	+	+	+	+	+	M	−	+	+	−	+	A	+	+	−	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, NOS																									
Malignant lymphoma, mixed type																									
Lymph nodes	−	+	+	+	−	M	+	−	−	+	−	A	−	+	+	−	−	−	+	+	−	+	−	−	−
Thymus	−	+	+	+	+	M	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	M	+	−	−	−	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	M	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
Liver	+	+	+	+	+	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Bile duct	+	+	+	+	+	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	N	+	N	N	M	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	−	+	+	+	M	−	−	−	−	−	A	−	+	−	−	−	−	−	−	−	−	−	−	−
Esophagus	−	+	+	+	+	M	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
Stomach	+	−	+	+	+	M	+	−	−	+	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
Small intestine	+	−	+	+	−	M	+	−	−	+	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
Large intestine	+	+	+	+	+	M	+	−	−	+	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
URINARY SYSTEM																									
Kidney	+	+	+	+	+	M	+	−	−	+	+	A	−	+	−	−	−	−	−	−	−	−	−	−	−
Urinary bladder	+	+	+	+	+	M	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
ENDOCRINE SYSTEM																									
Pituitary	−	+	+	+	+	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																			X						
Adrenal	+	+	+	+	+	M	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
Thyroid	+	+	+	+	+	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	−	−	+	+	−	M	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	+	+	+	M	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N
Uterus	+	+	+	+	+	M	+	+	+	+	+	A	+	−	+	−	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																									
Endometrial stromal polyp																							X		
Ovary	−	+	+	+	+	M	+	+	−	−	−	A	−	−	+	−	−	−	−	−	−	−	+	−	+
Papillary adenoma																									
Granulosa cell tumor																									
Choriocarcinoma		X																							
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	M	+	−	−	−	−	A	−	−	−	−	−	−	−	−	−	−	−	−	−
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	M	N	N	N	N	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N
Osteosarcoma							X																		
Osteosarcoma, metastatic											X														
Malignant lymphoma, NOS																						X			
Malignant lymphoma, undifferentiated type															X										
Malignant lymphoma, histiocytic type																									
Malignant lymphoma, mixed type									X					X						X					

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	0 1 5	0 1 6	0 1 7	0 1 8	0 1 9	0 2 0	0 2 3	0 2 5	0 2 6	0 2 7	0 2 9	0 3 0	0 3 2	0 3 3	0 3 4	0 3 5	0 3 7	0 3 8	0 3 9	0 4 0	0 4 1	0 4 3	0 4 4	0 4 7	0 4 8	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*48
Fibrosarcoma																									1
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma																									1
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
HEMATOPOIETIC SYSTEM																									
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Malignant lymphoma, NOS						X																			1
Malignant lymphoma, mixed type																						X			1
Lymph nodes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+	+	13
Thymus	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+	+	9
CIRCULATORY SYSTEM																									
Heart	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
DIGESTIVE SYSTEM																									
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma										X															1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*48
Pancreas	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
URINARY SYSTEM																									
Kidney	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenoma, NOS	X																								2
Adrenal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Thyroid	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Parathyroid	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*48
Uterus	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	44
Leiomyosarcoma															X										1
Endometrial stromal polyp																									1
Ovary	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	+	–	–	+	–	–	+	+	–	16
Papillary adenoma																						X			1
Granulosa cell tumor																			X						1
Choriocarcinoma																									1
NERVOUS SYSTEM																									
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*48
Osteosarcoma																									1
Osteosarcoma, metastatic																									1
Malignant lymphoma, NOS																									1
Malig. lymphoma, undifferentiated type												X													2
Malignant lymphoma, histiocytic type																		X							1
Malignant lymphoma, mixed type																			X						4

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE: HIGH DOSE

ANIMAL NUMBER	0555	0662	0884	0776	0773	0667	0775	0557	0881	0992	0666	0774	0551	0552	0553	0554	0556	0558	0559	0600	0661	0663	0864	0665	0668
WEEKS ON STUDY	0599	0683	0791	0881	0844	0991	0944	0996	0999	1022	1038	1033	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044	1044
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma, NOS																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						
Alveolar/bronchiolar carcinoma										X					X										
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Hemangiosarcoma				X																					
Malignant lymphoma, NOS																									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Thymus	−	+	+	+	+	−	+	−	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Esophagus	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−
ENDOCRINE SYSTEM																									
Pituitary	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS									X			X	X												
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma					X																				
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	+	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma		X																							
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Teratoma, benign			X																						
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS							X	X																X	X
Malignant lymphoma, undifferentiated type									X																
Malignant lymphoma, lymphocytic type					X																				
Malignant lymphoma, mixed type												X													
Neck, NOS																									
Hemangiosarcoma				X																					
Lumbar region																									
Hemangiosarcoma			X																						

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	069	070	071	072	077	078	079	080	082	083	085	086	087	088	089	090	091	093	094	095	096	097	098	099	100	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Papilloma, NOS		X																								1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X												X			X	X				5
Alveolar/bronchiolar carcinoma																										2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Malignant lymphoma, NOS													X													1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	–	+	+	+	+	–	+	–	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	–	+	39
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS					X		X																			5
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid	–	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+	+	+	–	+	–	39
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islet cell adenoma												X														1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	*50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Teratoma, benign																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, NOS																X										5
Malig. lymphoma, undifferentiated type																		X								2
Malignant lymphoma, lymphocytic type					X					X																3
Malignant lymphoma, mixed type								X													X					3
Neck, NOS																										
Hemangiosarcoma																										1
Lumbar region																										
Hemangiosarcoma																										1

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Control	12,500 ppm	25,000 ppm
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	2/49 (4%)	1/48 (2%)	5/50 (10%)
Adjusted Rates (b)	5.0%	2.9%	13.2%
Terminal Rates (c)	1/37 (3%)	1/35 (3%)	5/38 (13%)
Week of First Observation	102	104	104
Life Table Tests (d)	P=0.142	P=0.524N	P=0.226
Incidental Tumor Tests (d)	P=0.129	P=0.542N	P=0.204
Cochran-Armitage Trend Test (d)	P=0.139		
Fisher Exact Test (d)		P=0.508N	P=0.226
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	4/49 (8%)	1/48 (2%)	7/50 (14%)
Adjusted Rates (b)	9.7%	2.9%	17.8%
Terminal Rates (c)	2/37 (5%)	1/35 (3%)	6/38 (16%)
Week of First Observation	99	104	102
Life Table Tests (d)	P=0.188	P=0.206N	P=0.271
Incidental Tumor Tests (d)	P=0.151	P=0.222N	P=0.212
Cochran-Armitage Trend Test (d)	P=0.187		
Fisher Exact Test (d)		P=0.187N	P=0.274
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	1/49 (2%)	0/48 (0%)	3/50 (6%)
Adjusted Rates (b)	2.1%	0.0%	7.3%
Terminal Rates (c)	0/37 (0%)	0/35 (0%)	2/38 (5%)
Week of First Observation	84		84
Life Table Tests (d)	P=0.179	P=0.517N	P=0.305
Incidental Tumor Tests (d)	P=0.288	P=0.638N	P=0.434
Cochran-Armitage Trend Test (d)	P=0.181		
Fisher Exact Test (d)		P=0.505N	P=0.316
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	11/49 (22%)	5/48 (10%)	3/50 (6%)
Adjusted Rates (b)	28.9%	13.1%	7.6%
Terminal Rates (c)	10/37 (27%)	3/35 (9%)	2/38 (5%)
Week of First Observation	103	97	103
Life Table Tests (d)	P=0.012N	P=0.109N	P=0.020N
Incidental Tumor Tests (d)	P=0.017N	P=0.122N	P=0.025N
Cochran-Armitage Trend Test (d)	P=0.011N		
Fisher Exact Test (d)		P=0.093N	P=0.019N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	16/49 (33%)	10/48 (21%)	14/50 (28%)
Adjusted Rates (b)	39.5%	26.0%	32.2%
Terminal Rates (c)	13/37 (35%)	7/35 (20%)	9/38 (24%)
Week of First Observation	84	97	84
Life Table Tests (d)	P=0.361N	P=0.173N	P=0.406N
Incidental Tumor Tests (d)	P=0.401N	P=0.213N	P=0.434N
Cochran-Armitage Trend Test (d)	P=0.345N		
Fisher Exact Test (d)		P=0.139N	P=0.388N
Liver: Hepatocellular Adenoma			
Overall Rates (a)	8/49 (16%)	0/48 (0%)	0/50 (0%)
Adjusted Rates (b)	20.7%	0.0%	0.0%
Terminal Rates (c)	7/37 (19%)	0/35 (0%)	0/38 (0%)
Week of First Observation	99		
Life Table Tests (d)	P<0.001N	P=0.006N	P=0.005N
Incidental Tumor Tests (d)	P<0.001N	P=0.007N	P=0.006N
Cochran-Armitage Trend Test (d)	P<0.001N		
Fisher Exact Test (d)		P=0.003N	P=0.003N

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Control	12,500 ppm	25,000 ppm
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	3/49 (6%)	0/48 (0%)	0/50 (0%)
Adjusted Rates (b)	7.3%	0.0%	0.0%
Terminal Rates (c)	1/37 (3%)	0/35 (0%)	0/38 (0%)
Week of First Observation	99		
Life Table Tests (d)	P=0.040N	P=0.135N	P=0.125N
Incidental Tumor Tests (d)	P=0.054N	P=0.153N	P=0.167N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Test (d)		P=0.125N	P=0.117N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	10/49 (20%)	0/48 (0%)	0/50 (0%)
Adjusted Rates (b)	25.3%	0.0%	0.0%
Terminal Rates (c)	8/37 (22%)	0/35 (0%)	0/38 (0%)
Week of First Observation	99		
Life Table Tests (d)	P<0.001N	P=0.002N	P=0.001N
Incidental Tumor Tests (d)	P<0.001N	P=0.002N	P=0.002N
Cochran-Armitage Trend Test (d)	P<0.001N		
Fisher Exact Test (d)		P<0.001N	P<0.001N
Anterior Pituitary Gland: Adenoma			
Overall Rates (a)	11/48 (23%)	2/46 (4%)	5/49 (10%)
Adjusted Rates (b)	28.9%	5.9%	12.3%
Terminal Rates (c)	10/37 (27%)	2/34 (6%)	3/38 (8%)
Week of First Observation	103	104	99
Life Table Tests (d)	P=0.045N	P=0.012N	P=0.083N
Incidental Tumor Tests (d)	P=0.056N	P=0.013N	P=0.105N
Cochran-Armitage Trend Test (d)	P=0.043N		
Fisher Exact Test (d)		P=0.009N	P=0.078N
Anterior Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (a)	12/48 (25%)	2/46 (4%)	5/49 (10%)
Adjusted Rates (b)	30.8%	5.9%	12.3%
Terminal Rates (c)	10/37 (27%)	2/34 (6%)	3/38 (8%)
Week of First Observation	103	104	99
Life Table Tests (d)	P=0.026N	P=0.007N	P=0.054N
Incidental Tumor Tests (d)	P=0.035N	P=0.009N	P=0.075N
Cochran-Armitage Trend Test (d)	P=0.024N		
Fisher Exact Test (d)		P=0.005N	P=0.049N
Thyroid Gland: Follicular Cell Adenoma			
Overall Rates (a)	4/49 (8%)	0/47 (0%)	0/50 (0%)
Adjusted Rates (b)	10.0%	0.0%	0.0%
Terminal Rates (c)	2/37 (5%)	0/34 (0%)	0/38 (0%)
Week of First Observation	102		
Life Table Tests (d)	P=0.017N	P=0.076N	P=0.064N
Incidental Tumor Tests (d)	P=0.023N	P=0.086N	P=0.088N
Cochran-Armitage Trend Test (d)	P=0.015N		
Fisher Exact Test (d)		P=0.064N	P=0.056N
All Sites: Benign Tumors			
Overall Rates (a)	19/49 (39%)	5/48 (10%)	14/50 (28%)
Adjusted Rates (b)	46.1%	14.3%	32.8%
Terminal Rates (c)	15/37 (41%)	5/35 (14%)	10/38 (26%)
Week of First Observation	99	104	79
Life Table Tests (d)	P=0.149N	P=0.002N	P=0.197N
Incidental Tumor Tests (d)	P=0.152N	P=0.002N	P=0.209N
Cochran-Armitage Trend Test (d)	P=0.137N		
Fisher Exact Test (d)		P=0.001N	P=0.178N

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Control	12,500 ppm	25,000 ppm
All Sites: Malignant Tumors			
Overall Rates (a)	23/49 (47%)	14/48 (29%)	19/50 (38%)
Adjusted Rates (b)	53.1%	34.2%	40.0%
Terminal Rates (c)	17/37 (46%)	9/35 (26%)	10/38 (26%)
Week of First Observation	84	39	63
Life Table Tests (d)	P=0.252N	P=0.092N	P=0.290N
Incidental Tumor Tests (d)	P=0.217N	P=0.079N	P=0.265N
Cochran-Armitage Trend Test (d)	P=0.211N		
Fisher Exact Test (d)		P=0.055N	P=0.243N
All Sites: All Tumors			
Overall Rates (a)	34/49 (69%)	18/48 (38%)	26/50 (52%)
Adjusted Rates (b)	75.4%	44.3%	55.0%
Terminal Rates (c)	26/37 (70%)	13/35 (37%)	17/38 (45%)
Week of First Observation	84	39	63
Life Table Tests (d)	P=0.088N	P=0.008N	P=0.108N
Incidental Tumor Tests (d)	P=0.061N	P=0.003N	P=0.079N
Cochran-Armitage Trend Test (d)	P=0.053N		
Fisher Exact Test (d)		P=0.002N	P=0.059N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(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 lower incidence in a dosed group is indicated by (N).

TABLE D4a. HISTORICAL INCIDENCE OF HEPATOCELLULAR TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Physiological Research Laboratories			
Ephedrine sulfate	6/50	3/50	9/50
Phenylephrine hydrochloride	3/49	0/49	3/49
Oxytetracycline hydrochloride	5/50	2/50	6/50
TOTAL	14/149 (9.4%)	5/149 (3.4%)	18/149 (12.1%)
SD (b)	2.99%	3.06%	5.94%
Range (c)			
High	6/50	3/50	9/50
Low	3/49	0/49	3/49
Overall Historical Incidence			
TOTAL	97/2,033 (4.8%)	(d) 83/2,033 (4.1%)	177/2,033 (8.7%)
SD (b)	4.14%	2.61%	4.75%
Range (c)			
High	9/49	5/50	10/49
Low	0/50	0/50	0/50

(a) Data as of August 7, 1986, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) One hepatoblastoma was also observed.

TABLE D4b. HISTORICAL INCIDENCE OF ANTERIOR PITUITARY GLAND TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma (b)	Carcinoma (c)	Adenoma or Carcinoma (b,c)
Historical Incidence at Physiological Research Laboratories			
Ephedrine sulfate	8/48	2/48	10/48
Phenylephrine hydrochloride	0/48	0/48	0/48
Oxytetracycline hydrochloride	13/50	3/50	16/50
TOTAL	21/146 (14.4%)	5/146 (3.4%)	26/146 (17.8%)
SD (d)	13.17%	3.07%	16.24%
Range (e)			
High	13/50	3/50	16/50
Low	0/48	0/48	0/48
Overall Historical Incidence			
TOTAL	192/1,764 (10.9%)	12/1,764 (0.7%)	204/1,764 (11.6%)
SD (d)	9.47%	1.44%	9.67%
Range (e)			
High	12/40	3/50	16/50
Low	0/48	0/49	0/48

(a) Data as of August 7, 1986, for studies of at least 104 weeks
(b) Includes adenomas, NOS, and chromophobe adenomas
(c) Includes carcinomas, NOS, adenocarcinomas, NOS, and chromophobe carcinomas
(d) Standard deviation
(e) 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 FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals missing	1	1	
Animals necropsied	49	48	50
Animals examined histopathologically	49	48	50
INTEGUMENTARY SYSTEM			
*Skin	(49)	(48)	(50)
Infection, bacterial	1 (2%)		
Alopecia	1 (2%)		
RESPIRATORY SYSTEM			
#Nasal mucosa	(46)	(0)	(45)
Multiple cysts			1 (2%)
#Tracheal mucosa	(49)	(4)	(49)
Inflammation, acute			1 (2%)
#Lung	(49)	(48)	(50)
Congestion, NOS		1 (2%)	
Hemorrhage	1 (2%)	1 (2%)	
Pneumonia, interstitial chronic		6 (13%)	4 (8%)
Amyloidosis		1 (2%)	
Calcification, focal		1 (2%)	
Alveolar macrophages	1 (2%)		3 (6%)
Hyperplasia, alveolar epithelium			1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(48)	(50)
Hyperplasia, lymphoid			3 (6%)
#Bone marrow	(48)	(4)	(50)
Fibrosis, multifocal			1 (2%)
Hyperplasia, granulocytic	1 (2%)		3 (6%)
Hypoplasia, hematopoietic			1 (2%)
#Spleen	(49)	(45)	(49)
Angiectasis		1 (2%)	1 (2%)
Hyperplasia, lymphoid	18 (37%)	20 (44%)	15 (31%)
Hematopoiesis	6 (12%)	2 (4%)	3 (6%)
#Lymph node	(48)	(13)	(49)
Amyloidosis		1 (8%)	
#Mandibular lymph node	(48)	(13)	(49)
Hyperplasia, lymphoid	2 (4%)		2 (4%)
#Mesenteric lymph node	(48)	(13)	(49)
Angiectasis	2 (4%)		
Hyperplasia, reticulum cell			1 (2%)
Hyperplasia, lymphoid	5 (10%)		1 (2%)
#Lung	(49)	(48)	(50)
Hyperplasia, lymphoid	1 (2%)	5 (10%)	1 (2%)
#Salivary gland	(48)	(5)	(47)
Hyperplasia, lymphoid	7 (15%)	1 (20%)	6 (13%)
#Liver	(49)	(48)	(50)
Hyperplasia, lymphoid	5 (10%)	8 (17%)	3 (6%)
#Pancreas	(49)	(6)	(49)
Hyperplasia, lymphoid			1 (2%)
#Glandular stomach	(49)	(6)	(50)
Hyperplasia, lymphoid			1 (2%)
#Cecum	(49)	(7)	(50)
Hyperplasia, lymphoid	1 (2%)		
#Kidney	(49)	(9)	(50)
Hyperplasia, lymphoid	8 (16%)	2 (22%)	13 (26%)
#Urinary bladder	(49)	(5)	(48)
Hyperplasia, lymphoid	1 (2%)		2 (4%)

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Thymus	(41)	(9)	(39)
Necrosis, NOS		1 (11%)	
Hyperplasia, lymphoid	1 (2%)		2 (5%)
CIRCULATORY SYSTEM			
#Heart	(48)	(6)	(50)
Endocarditis, bacterial	1 (2%)	1 (17%)	
#Myocardium	(48)	(6)	(50)
Inflammation, chronic focal		1 (17%)	
Calcification, NOS			1 (2%)
#Liver	(49)	(48)	(50)
Thrombosis, NOS	1 (2%)		
DIGESTIVE SYSTEM			
#Salivary gland	(48)	(5)	(47)
Atrophy, NOS			1 (2%)
#Liver	(49)	(48)	(50)
Multiple cysts	1 (2%)		
Inflammation, acute/chronic			3 (6%)
Inflammation, chronic focal			1 (2%)
Necrosis, NOS		1 (2%)	
Necrosis, focal		1 (2%)	
Necrosis, central			1 (2%)
Pigmentation, NOS		1 (2%)	
Cytoplasmic vacuolization		2 (4%)	1 (2%)
Focal cellular change	1 (2%)		
Angiectasis	1 (2%)		
*Gallbladder	(49)	(48)	(50)
Distention	1 (2%)		
#Pancreas	(49)	(6)	(49)
Cystic ducts		1 (17%)	1 (2%)
Inflammation, chronic			1 (2%)
Atrophy, NOS		1 (17%)	
#Pancreatic acinus	(49)	(6)	(49)
Hypertrophy, focal			1 (2%)
#Glandular stomach	(49)	(6)	(50)
Cyst, NOS			4 (8%)
Multiple cysts	3 (6%)		2 (4%)
Eosinophilic leukocytic infiltrate	1 (2%)		
Inflammation, chronic	4 (8%)		1 (2%)
Inflammation, chronic focal	2 (4%)		
Calcification, NOS		1 (17%)	
Pigmentation, NOS	1 (2%)		
Hyperplasia, focal			1 (2%)
#Forestomach	(49)	(6)	(50)
Inflammation, acute necrotizing			1 (2%)
Inflammation, chronic focal			1 (2%)
Reaction, foreign body	1 (2%)		
Hyperplasia, epithelial	1 (2%)		
Hyperkeratosis		2 (33%)	

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM			
#Kidney	(49)	(9)	(50)
Cast, NOS	1 (2%)		
Glomerulonephritis, NOS		1 (11%)	1 (2%)
Pyelonephritis, NOS	1 (2%)		
Inflammation, chronic		1 (11%)	
Degeneration, hyaline	1 (2%)		
Amyloidosis		2 (22%)	
Calcification, focal	3 (6%)		
#Kidney/medulla	(49)	(9)	(50)
Inflammation, acute		1 (11%)	
#Kidney/tubule	(49)	(9)	(50)
Pigmentation, NOS			1 (2%)
Regeneration, NOS	3 (6%)		
ENDOCRINE SYSTEM			
#Anterior pituitary	(48)	(46)	(49)
Cyst, NOS	1 (2%)	3 (7%)	3 (6%)
Hyperplasia, focal	11 (23%)	16 (35%)	7 (14%)
Angiectasis	3 (6%)		
#Adrenal/capsule	(49)	(5)	(50)
Hyperplasia, stromal	45 (92%)	3 (60%)	45 (90%)
#Adrenal cortex	(49)	(5)	(50)
Cyst, NOS			1 (2%)
Inflammation, acute focal			1 (2%)
Degeneration, lipoid	5 (10%)		2 (4%)
Hyperplasia, focal	2 (4%)		
#Adrenal medulla	(49)	(5)	(50)
Focal cellular change	1 (2%)		
#Thyroid	(49)	(47)	(50)
Cystic follicles	1 (2%)	1 (2%)	7 (14%)
Atrophy, NOS		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(49)	(48)	(50)
Multiple cysts	3 (6%)		2 (4%)
*Clitoral gland	(49)	(48)	(50)
Cystic ducts		1 (2%)	
#Uterus	(49)	(44)	(50)
Dilatation, NOS		1 (2%)	
Hemorrhage, chronic	1 (2%)		
Inflammation, chronic suppurative	2 (4%)		1 (2%)
Hyperplasia, stromal		1 (2%)	
#Uterus/endometrium	(49)	(44)	(50)
Hyperplasia, cystic	41 (84%)	38 (86%)	41 (82%)
#Fallopian tube	(49)	(44)	(50)
Multiple cysts	1 (2%)		
#Ovary	(48)	(16)	(50)
Cyst, NOS	9 (19%)	5 (31%)	8 (16%)
Hematoma, organized		1 (6%)	
Hemorrhagic cyst		1 (6%)	
Inflammation, necrotizing		1 (6%)	
Abscess, NOS			1 (2%)

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE (Continued)

	Untreated Control	Low Dose	High Dose
NERVOUS SYSTEM			
#Brain/meninges	(49)	(6)	(50)
Inflammation with fibrosis	1 (2%)		
Perivascular cuffing	2 (4%)		1 (2%)
#Brain	(49)	(6)	(50)
Spongiosis		1 (17%)	
Perivascular cuffing			1 (2%)
Degeneration, myelin			1 (2%)
Calcification, focal	18 (37%)	1 (17%)	15 (30%)
SPECIAL SENSE ORGANS			
*Eye	(49)	(48)	(50)
Cataract			1 (2%)
*Eye/cornea	(49)	(48)	(50)
Inflammation, chronic			1 (2%)
MUSCULOSKELETAL SYSTEM			
*Femur	(49)	(48)	(50)
Fibrosis, multifocal			1 (2%)
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(48)	(50)
Inflammation, necrotizing granulomatous	1 (2%)		
Pituitary fossa			
Inflammation with fibrosis			1
Adipose tissue			
Necrosis, fat	1		
SPECIAL MORPHOLOGY SUMMARY			
Animal missing/no necropsy	1	1	
Auto/necropsy/histo perf	1		
Autolysis/no necropsy		1	

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX E

GENETIC TOXICOLOGY OF

TETRACYCLINE HYDROCHLORIDE

TABLE E1. MUTAGENICITY OF TETRACYCLINE HYDROCHLORIDE IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/Plate (b) −S9 Trial 1	−S9 Trial 2	+S9 (hamster) Trial 1	+S9 (hamster) Trial 2	+S9 (rat) Trial 1	+S9 (rat) Trial 2
TA100	0	105 ± 7.7	109 ± 2.9	121 ± 4.5	93 ± 4.0	118 ± 4.7	103 ± 5.8
	0.03	--	100 ± 6.3	--	116 ± 11.6	--	99 ± 6.4
	0.1	113 ± 8.8	93 ± 2.9	121 ± 3.1	128 ± 9.6	125 ± 6.4	103 ± 10.3
	0.3	101 ± 2.0	115 ± 10.6	117 ± 4.5	121 ± 7.5	94 ± 8.7	99 ± 9.5
	1	49 ± 7.5	82 ± 7.4	69 ± 10.9	101 ± 7.0	77 ± 8.0	86 ± 2.9
	3	5 ± 1.0	(c) 10 ± 4.0	5 ± 2.0	(c) 20 ± 3.3	5 ± 1.2	46 ± 8.8
	10	Toxic	--	Toxic	--	6 ± 3.5	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	419 ± 12.6	336 ± 7.9	778 ± 10.2	991 ± 7.8	495 ± 23.2	392 ± 5.5
TA1535	0	24 ± 4.7	22 ± 1.9	8 ± 1.9	9 ± 2.7	11 ± 2.7	10 ± 4.0
	0.03	--	14 ± 2.7	--	3 ± 0.9	--	14 ± 0.7
	0.1	15 ± 1.8	21 ± 1.8	12 ± 2.6	4 ± 1.5	15 ± 1.2	14 ± 2.3
	0.3	16 ± 1.9	18 ± 4.7	8 ± 2.9	4 ± 0.3	15 ± 1.5	5 ± 1.5
	1	11 ± 2.7	15 ± 0.3	8 ± 2.0	3 ± 1.0	10 ± 1.2	11 ± 1.2
	3	Toxic	(c) 6 ± 0.7	2 ± 0.7	2 ± 0.3	5 ± 1.2	7 ± 1.0
	10	Toxic	--	Toxic	--	Toxic	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	379 ± 22.3	334 ± 14.3	356 ± 53.3	337 ± 24.8	120 ± 13.2	232 ± 8.0
TA1537	0	8 ± 1.5	8 ± 2.5	6 ± 0.3	6 ± 1.2	16 ± 2.5	15 ± 1.5
	0.03	--	6 ± 3.8	--	7 ± 2.9	--	12 ± 4.3
	0.1	11 ± 1.2	4 ± 1.2	9 ± 3.7	6 ± 3.5	12 ± 1.8	14 ± 1.2
	0.3	9 ± 3.0	6 ± 3.5	8 ± 0.7	6 ± 3.5	15 ± 0.9	11 ± 2.8
	1	6 ± 0.3	4 ± 1.5	5 ± 1.2	5 ± 0.3	13 ± 2.2	8 ± 0.7
	3	Toxic	(c) 3 ± 1.0	Toxic	(c) 2 ± 0.7	5 ± 1.5	8 ± 2.3
	10	Toxic	--	Toxic	--	6 ± 1.8	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	277 ± 25.1	177 ± 7.0	454 ± 17.6	248 ± 2.3	204 ± 14.8	121 ± 12.5
TA98	0	15 ± 1.5	13 ± 0.7	27 ± 3.4	35 ± 4.4	32 ± 4.1	29 ± 0.7
	0.03	--	22 ± 5.2	--	27 ± 0.7	--	32 ± 3.1
	0.1	16 ± 4.3	15 ± 1.5	24 ± 3.5	19 ± 3.5	38 ± 3.2	34 ± 3.3
	0.3	13 ± 2.8	20 ± 2.3	25 ± 1.0	26 ± 3.6	39 ± 5.8	39 ± 3.2
	1	4 ± 0.6	11 ± 2.2	17 ± 4.7	22 ± 2.5	17 ± 2.3	30 ± 0.9
	3	Toxic	(c) 4 ± 0.9	Toxic	(c) 1 ± 0.3	8 ± 1.0	17 ± 2.2
	10	Toxic	--	Toxic	--	10 ± 2.7	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (d)	730 ± 18.6	693 ± 16.6	477 ± 29.8	858 ± 48.1	401 ± 33.1	236 ± 16.8

(a) Study performed at SRI International. The detailed protocol is presented by Haworth et al. (1983). Cells and study compound or solvent (distilled water) 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 E2. INDUCTION OF TRIFLUOROTHYMIDINE RESISTANCE IN MOUSE L5178Y LYMPHOMA CELLS BY TETRACYCLINE HYDROCHLORIDE (a,b)

Compound	Concentration (µl or µg/ml)	Cloning Efficiency (percent)	Relative Total Growth (percent)	Tft-Resistant Cells	Mutant Fraction (c)
-S9					
Trial 1					
Distilled water (d)		94.3 ± 5.9	100.3 ± 4.4	111.3 ± 4.4	40.3 ± 4.3
Tetracycline hydrochloride	40	96.0 ± 11.4	87.3 ± 5.5	118.0 ± 5.5	41.7 ± 4.1
	50	83.7 ± 1.2	82.0 ± 9.2	105.0 ± 6.6	41.7 ± 2.7
	60	87.0 ± 10.7	82.3 ± 0.3	116.0 ± 18.0	44.7 ± 5.2
	80	85.0 ± 5.6	67.0 ± 1.2	89.7 ± 12.6	35.0 ± 3.2
	100	78.0 ± 3.0	45.7 ± 7.7	111.3 ± 4.8	47.7 ± 3.5
	200	Lethal	--	--	--
Methyl methanesulfonate (e)	5	65.3 ± 3.4	63.0 ± 9.8	792.7 ± 93.3	(f) 401.3 ± 25.9
Trial 2					
Distilled water (d)		108.8 ± 3.7	100.3 ± 2.8	103.8 ± 10.5	31.8 ± 2.8
Tetracycline hydrochloride	50	87.3 ± 8.0	71.0 ± 7.8	81.3 ± 4.9	31.3 ± 1.2
	60	90.3 ± 4.3	82.3 ± 1.2	94.7 ± 1.2	35.0 ± 1.0
	(e) 80	87.5 ± 0.5	74.5 ± 3.5	80.5 ± 2.5	30.5 ± 0.5
	100	96.0 ± 5.9	61.0 ± 4.2	100.7 ± 8.4	35.3 ± 3.2
	150	85.7 ± 7.2	31.0 ± 2.6	92.7 ± 2.6	36.7 ± 3.9
	200	68.7 ± 4.7	6.3 ± 0.3	127.3 ± 10.2	(f) 61.7 ± 4.3
Methyl methanesulfonate (e)	5	65.3 ± 3.8	39.3 ± 2.4	547.3 ± 17.5	(f) 279.7 ± 13.1
Trial 3					
Distilled water (d)		66.0 ± 6.2	100.3 ± 4.7	56.0 ± 5.8	28.8 ± 3.8
Tetracycline hydrochloride	25	94.3 ± 11.1	131.3 ± 10.9	64.0 ± 5.3	22.7 ± 1.7
	50	89.0 ± 7.2	113.7 ± 3.3	63.7 ± 3.9	24.0 ± 3.0
	75	95.3 ± 7.1	113.3 ± 9.6	52.3 ± 6.2	19.0 ± 3.2
	100	90.3 ± 7.7	75.3 ± 4.4	67.0 ± 6.7	25.3 ± 3.7
	150	66.7 ± 3.5	28.7 ± 3.0	71.3 ± 12.9	36.7 ± 8.7
	(e) 200	74.0 ± 8.0	10.0 ± 3.0	71.5 ± 7.5	33.0 ± 7.0
	300	Lethal	--	--	--
Methyl methanesulfonate (e)	5	63.7 ± 5.8	68.7 ± 5.8	275.0 ± 32.5	(f) 143.3 ± 4.3
+S9 (induced) (g)					
Trial 1					
Distilled water (d)		96.8 ± 2.6	100.0 ± 8.0	93.0 ± 9.4	32.0 ± 2.9
Tetracycline hydrochloride	10	84.3 ± 7.1	77.0 ± 0.6	73.0 ± 10.5	28.7 ± 1.8
	20	82.0 ± 7.9	79.7 ± 8.4	45.0 ± 2.5	18.3 ± 0.9
	30	87.0 ± 9.3	60.0 ± 3.6	64.3 ± 6.3	25.0 ± 1.7
	40	94.0 ± 7.0	83.3 ± 8.5	77.3 ± 4.3	27.7 ± 0.9
	50	84.0 ± 4.6	57.0 ± 3.6	60.0 ± 3.2	24.0 ± 2.3
	60	98.0 ± 6.0	48.7 ± 4.3	72.3 ± 16.0	24.3 ± 4.4
Methylcholanthrene	2.5	75.7 ± 8.9	41.7 ± 3.3	505.3 ± 57.6	(f) 224.0 ± 16.4

TABLE E2. INDUCTION OF TRIFLUOROTHYMIDINE RESISTANCE IN MOUSE L5178Y LYMPHOMA CELLS BY TETRACYCLINE HYDROCHLORIDE (Continued)

Compound	Concentration (µl or µg/ml)	Cloning Efficiency (percent)	Relative Total Growth (percent)	Tft-Resistant Cells	Mutant Fraction (c)
+S9 (induced) (Continued)					
Trial 2					
Distilled water (d)		91.5 ± 1.9	100.0 ± 4.7	89.5 ± 4.4	33.0 ± 2.0
Tetracycline hydrochloride	(e) 40	87.5 ± 4.5	60.5 ± 5.5	104.0 ± 9.0	39.5 ± 1.5
	50	107.3 ± 1.8	61.7 ± 3.0	76.3 ± 7.9	24.0 ± 3.0
	60	105.7 ± 1.2	50.3 ± 4.6	93.0 ± 14.6	29.3 ± 4.9
	80	102.3 ± 7.9	24.7 ± 0.7	114.3 ± 8.4	38.0 ± 4.5
	100	85.0 ± 7.0	13.7 ± 1.9	104.7 ± 9.5	41.0 ± 0.6
	120	87.3 ± 8.0	10.0 ± 1.2	123.7 ± 13.0	48.7 ± 8.7
Methylcholanthrene	2.5	59.7 ± 9.1	19.3 ± 3.0	555.7 ± 16.0	(f) 325.7 ± 47.4
+S9 (noninduced) (h)					
Trial 1					
Distilled water (d)		73.0 ± 6.0	100.0 ± 6.8	75.0 ± 14.8	34.5 ± 7.4
Tetracycline hydrochloride	20	53.0 ± 4.6	96.0 ± 9.8	59.7 ± 2.0	38.0 ± 1.7
	30	61.7 ± 3.8	72.0 ± 9.1	87.7 ± 4.7	47.7 ± 3.4
	40	57.0 ± 4.7	60.7 ± 9.8	71.7 ± 15.3	44.0 ± 13.1
	50	Lethal	--	--	--
	60	47.0 ± 9.3	28.3 ± 4.3	87.0 ± 3.5	(f) 65.7 ± 10.1
	(e) 80	46.5 ± 2.5	14.5 ± 2.5	100.0 ± 2.0	(f) 72.5 ± 2.5
	100	Lethal			
Methylcholanthrene	3.5	83.7 ± 9.8	55.3 ± 13.2	539.0 ± 35.6	(f) 218.0 ± 14.0
Trial 2					
Distilled water (d)		103.8 ± 6.7	100.0 ± 2.2	118.3 ± 4.1	38.3 ± 1.4
Tetracycline hydrochloride	40	97.7 ± 3.8	56.3 ± 4.9	93.3 ± 10.4	31.7 ± 3.0
	50	94.0 ± 5.5	55.3 ± 4.3	124.0 ± 7.4	44.3 ± 1.9
	60	97.0 ± 5.1	38.7 ± 3.4	113.3 ± 11.4	39.7 ± 4.8
	80	110.0 ± 5.0	21.3 ± 1.2	118.3 ± 21.1	35.7 ± 5.4
	100	89.3 ± 2.9	15.7 ± 1.2	133.0 ± 9.3	49.7 ± 2.7
	120	78.7 ± 4.2	10.7 ± 0.9	148.7 ± 5.4	(f) 63.3 ± 5.0
Methylcholanthrene	3.5	93.0 ± 4.2	56.3 ± 5.6	580.3 ± 26.7	(f) 208.7 ± 13.9
Trial 3					
Distilled water (d)		98.5 ± 5.2	100.3 ± 5.4	119.8 ± 5.0	41.3 ± 3.1
Tetracycline hydrochloride	(e) 40	78.0 ± 0.0	61.0 ± 1.0	82.0 ± 17.0	35.0 ± 7.0
	(d) 60	74.5 ± 4.5	32.5 ± 3.6	71.5 ± 4.3	32.3 ± 1.7
	(d) 80	80.5 ± 2.6	19.8 ± 0.8	103.0 ± 25.4	42.8 ± 11.2
	(d) 100	92.0 ± 3.5	15.5 ± 1.0	136.8 ± 14.5	49.5 ± 4.5
	(d) 120	69.5 ± 3.3	8.8 ± 0.8	135.8 ± 16.3	64.3 ± 4.7
Methylcholanthrene	(e) 3.5	99.0 ± 3.0	75.0 ± 5.0	652.5 ± 58.5	(f) 219.5 ± 13.5

TABLE E2. INDUCTION OF TRIFLUOROTHYMIDINE RESISTANCE IN MOUSE L5178Y LYMPHOMA CELLS BY TETRACYCLINE HYDROCHLORIDE (Continued)

(a) Study performed at Litton Bionetics, Inc. The experimental protocol is presented in detail by Myhr et al. (1985) and follows the basic format of Clive et al. (1979). The highest dose of study compound is determined by solubility or toxicity and may not exceed 5 mg/ml. All doses are tested in triplicate; the average for the three tests is presented in the table. Cells (6×10^5/ml) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine (Tft) for selection of Tft-resistant cells, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.
(b) Mean ± standard error from replicate trials of approximately 1×10^6 cells each. All data are evaluated statistically for both trend and peak response ($P<0.05$ for at least one of the three highest dose sets). Both responses must be significantly ($P<0.05$) positive for a chemical to be considered capable of inducing Tft resistance. If only one of these responses is significant, the call is "equivocal"; the absence of both trend and peak response results in a "negative" call.
(c) Mutant fraction (frequency) is a ratio of the Tft-resistant cells to the cloning efficiency, divided by 3 (to arrive at MF per 1×10^6 cells treated); MF = mutant fraction.
(d) Data presented are the average of four tests.
(e) Data presented are the average of two tests.
(f) Significant positive response; occurs when the relative mutant fraction (average MF of treated culture/average MF of solvent control) is greater than or equal to 1.6.
(g) Tests conducted with metabolic activation were performed as described in (a) except that S9, prepared from the liver of Aroclor 1254-induced F344 rats, was added at the same time as the study chemical and/or solvent.
(h) Tests performed as in (g) except that S9 was prepared from the liver of noninduced F344 rats.

TABLE E3. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY TETRACYCLINE HYDROCHLORIDE (a)

Compound	Dose (µg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hours in BrdU	Relative SCEs/Cell (percent) (b)
-S9 (c)								
Trial 1--Summary: Negative								
Medium		50	1,044	443	0.42	8.9	25.7	--
Tetracycline hydrochloride								
	10	50	1,040	470	0.45	9.4	25.7	105.6
	24.8	50	1,036	526	0.51	10.5	(d) 33.2	118.0
	49.9	0	--	--	--	--	--	--
Mitomycin C	0.001	50	1,040	757	0.73	15.1	25.7	169.7
	0.01	5	105	281	2.68	56.2	25.7	631.5
Trial 2--Summary: Negative								
Medium		50	1,034	404	0.39	8.1	25.5	--
Tetracycline hydrochloride								
	5	50	1,036	423	0.41	8.5	25.5	104.9
	10	50	1,029	386	0.38	7.7	25.5	95.1
	24.9	50	1,037	400	0.39	8.0	(d) 32.2	98.8
	40.2	0	--	--	--	--	(d) 32.2	--
Mitomycin C	0.001	50	1,032	565	0.55	11.3	25.5	139.5
	0.01	5	105	189	1.80	37.8	25.5	466.7
+S9 (e)								
Trial 1--Summary: Negative								
Medium		50	1,041	451	0.43	9.0	25.7	--
Tetracycline hydrochloride								
	302.4	50	1,023	516	0.50	10.3	25.7	114.4
	402	50	1,039	527	0.51	10.5	(d) 33.2	116.7
	504	50	1,038	524	0.50	10.5	(d) 33.2	116.7
	600	0	--	--	--	--	(d) 33.2	--
Cyclophosphamide	0.4	50	1,040	632	0.61	12.6	25.7	140.0
	2	5	105	186	1.77	37.2	25.7	413.3

(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 (medium) as described in (c) or (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) SCEs/cell in treated culture expressed as a percent of the SCEs/cell in the control culture
(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. Then cells were 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.

TABLE E4. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY TETRACYCLINE HYDROCHLORIDE (a)

Trial 1					Trial 2				
Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
−S9 (b)--Harvest time 10.5 h					−S9 (b)--Harvest time 10.5 h				
Medium					Medium				
	100	0	0.00	0		100	1	0.01	1
	100	5	0.05	5		100	2	0.02	2
Tetracycline hydrochloride					Tetracycline hydrochloride				
39.9	100	4	0.04	4	150	100	0	0.00	0
49.7	100	3	0.03	3	200	100	1	0.01	1
70	100	1	0.01	1	300	100	0	0.00	0
					400	0	--	--	--
Summary: Negative					Summary: Negative				
Mitomycin C					Mitomycin C				
0.15	100	8	0.08	8	0.15	100	11	0.11	10
0.5	25	7	0.28	24	0.5	50	11	0.22	18
+S9 (c)--Harvest time 20.5 h (d)					+S9 (c)--Harvest time 21.0 h (d)				
Medium					Medium				
	100	1	0.01	1		100	1	0.01	1
Tetracycline hydrochloride					Tetracycline hydrochloride				
1,000	100	2	0.02	2	2,260	100	1	0.01	1
1,500	100	2	0.02	2	2,500	100	0	0.00	0
2,000	200	14	0.07	7	2,750	100	1	0.01	1
2,500	0	--	--	--					
Summary: Weakly positive					Summary: Negative				
Cyclophosphamide					Cyclophosphamide				
6.25	100	15	0.15	12	6.25	100	8	0.08	7
12.5	25	20	0.80	52	12.5	26	9	0.35	28

(a) Study performed at Litton Bionetics, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is presented by Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (medium) as indicated in (b) or (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, Chinese hamster ovary cells were incubated with study compound or solvent (medium) 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 (medium) 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 chemically induced cell cycle delay, incubation time before addition of colcemid was lengthened to provide sufficient metaphases at harvest.

TABLE E5. INDUCTION OF SEX-LINKED RECESSIVE LETHAL MUTATIONS IN DROSOPHILA BY TETRACYCLINE HYDROCHLORIDE (a)

Route of Exposure	Dose (ppm)	Incidence of Deaths (percent)	Incidence of Sterility (percent)	No. of Lethals/No. of X Chromosomes Tested			Overall Total (b)
				Mating 1	Mating 2	Mating 3	
Injection	5,000	22	24	1/987	1/971	0/978	2/2,936 (0.07%)
	0			1/1,022	0/1,017	0/991	1/3,030 (0.03%)
Injection	5,300	35	40	0/1,320	1/760	2/691	3/2,771 (0.11%)
	0			0/948	1/997	2/848	3/2,793 (0.11%)
Feeding	9,005	0	0	3/2,004	1/1,896	1/2,093	5/5,993 (0.08%)
	0			1/2,077	0/1,635	2/2,010	3/5,722 (0.05%)

(a) Study performed at Brown University. A detailed protocol of the sex-linked recessive lethal assay is presented in Zimmering et al. (1985). Exposure by feeding was done by allowing 24-hour-old Canton-S males to feed for 3 days on a solution of the study chemical dissolved in 5% sucrose. In the injection experiments, 24-hour-old Canton-S males were treated with a solution of the chemical dissolved in 0.7% saline and allowed 24 hours to recover. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three broods of 3, 2, and 2 days; sample sperm from successive matings were treated as spermatozoa (mating 1), spermatids (mating 2), and spermatocytes (mating 3). F_1 heterozygous females were crossed to their siblings and placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters; no clusters were found. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. Results were not significant at the 5% level (Margolin et al., 1983).
(b) Combined total of number of lethal mutations/number of X chromosomes tested for three mating trials

APPENDIX F

SENTINEL ANIMAL PROGRAM

APPENDIX F. SENTINEL ANIMAL PROGRAM

Methods

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

Fifteen B6C3F$_1$ mice and 15 F344/N rats of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group were killed at 6, 12, and 18 months on study. 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) Ectro (infectious ectromelia) Sendai (6,12,24 mo)	M. Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus) Sendai (18 mo)	MHV (mouse hepatitis virus) (12,18,24 mo)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai (6,12,24 mo)	RCV (rat coronavirus) Sendai (18 mo)	

Results

Results are presented in Table F1.

TABLE F1. MURINE VIRUS ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE (a)

Interval (months)	Number of Animals	Positive Serologic Reaction for
RATS		
6	--	None positive
12	10/10	Sendai
18	10/10	Sendai
24	8/10	Sendai
MICE		
6	--	None positive
12	--	None positive
18	--	None positive
24	1/10	Sendai

(a) Blood samples were taken from sentinel animals at 6, 12, and 18 months after the start of dosing and from the control animals just before they were killed; samples were sent to Microbiological Associates (Bethesda, MD) for determination of antibody titers.

APPENDIX G

FEED AND COMPOUND CONSUMPTION BY RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF TETRACYCLINE HYDROCHLORIDE

TABLE G1. FEED AND COMPOUND CONSUMPTION BY MALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

Week	Control		12,500 ppm			25,000 ppm		
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
4	15	255	15	245	765	15	239	1,569
8	17	327	16	310	645	17	306	1,389
13	17	377	16	366	546	18	364	1,236
19	18	402	18	399	564	18	393	1,145
24	16	430	15	420	446	16	415	964
29	16	452	16	437	458	16	433	924
32	15	452	15	441	425	15	432	868
37	17	461	15	450	417	16	444	901
41	16	468	15	459	408	16	453	883
45	17	478	16	467	428	17	461	922
50	15	478	15	471	398	16	466	858
54	16	476	15	471	398	15	465	806
58	13	469	13	468	347	13	457	711
63	15	474	12	471	318	11	462	595
67	14	484	13	474	343	14	465	753
71	15	462	15	460	408	16	448	893
76	15	452	15	463	405	16	439	911
80	14	450	14	453	386	14	439	797
84	15	444	13	441	368	14	436	803
88	14	433	13	437	372	14	425	824
93	14	449	13	438	371	13	431	754
97	13	438	18	440	511	13	425	765
101	12	424	11	419	328	12	418	718
Mean	15.2	436	14.7	430	437	15.0	422	913
SD (c)	1.5		1.7		107	1.8		226
CV(d)	9.9		11.6		24.5	12.0		24.8

(a) Grams of feed removed from the feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of tetracycline hydrochloride consumed per day per kilogram of body weight
(c) Standard deviation
(d) Coefficient of variation = (standard deviation/mean) × 100

TABLE G2. FEED AND COMPOUND CONSUMPTION BY FEMALE RATS IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

Week	Control		12,500 ppm			25,000 ppm		
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
4	11	164	12	166	904	11	162	1,698
8	10	194	10	195	641	11	185	1,486
13	11	211	11	214	643	11	210	1,310
19	11	219	11	224	614	11	217	1,267
24	10	228	10	235	532	10	225	1,111
29	10	234	9	241	467	10	234	1,068
32	10	240	10	244	512	10	235	1,064
37	10	245	10	252	496	10	244	1,025
41	11	250	11	260	529	11	248	1,109
45	11	258	11	266	517	9	254	886
50	11	268	11	273	504	11	259	1,062
54	11	270	11	279	493	11	263	1,046
58	11	277	10	287	436	10	269	929
63	12	285	11	294	468	10	273	916
67	10	295	10	305	410	10	282	887
71	12	299	12	310	484	12	287	1,045
76	11	306	12	317	473	12	298	1,007
80	10	298	10	307	407	10	290	862
84	12	294	12	312	481	12	295	1,017
88	11	300	11	319	431	11	294	935
93	11	315	11	328	419	11	302	911
97	11	318	11	330	417	11	306	899
101	10	317	9	319	353	10	304	822
Mean	10.8	265	10.7	273	506	10.7	258	1,059
SD (c)	0.7		0.9		113	0.8		209
CV (d)	6.5		8.4		22.3	7.5		19.7

(a) Grams of feed removed from the feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of tetracycline hydrochloride consumed per day per kilogram of body weight
(c) Standard deviation
(d) Coefficient of variation = (standard deviation/mean) × 100

TABLE G3. FEED AND COMPOUND CONSUMPTION BY MALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

Week	Control		12,500 ppm			25,000 ppm		
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
3	3	25.4	3	24.0	1,563	3	23.5	3,191
9	4	30.3	4	27.9	1,792	4	27.6	3,623
13	4	32.5	4	29.7	1,684	4	28.8	3,472
17	2	32.6	4	30.2	1,656	4	29.8	3,356
22	4	34.0	4	31.5	1,587	4	30.6	3,268
26	4	35.5	4	32.3	1,548	4	31.5	3,175
31	4	36.5	4	32.5	1,538	4	31.9	3,135
35	4	35.8	4	32.5	1,538	4	32.0	3,125
39	4	37.9	4	34.1	1,466	4	32.4	3,086
43	4	37.5	4	33.9	1,475	4	32.3	3,096
48	4	37.9	4	34.3	1,458	4	33.2	3,012
52	4	37.6	4	34.3	1,458	4	33.2	3,012
57	3	37.9	3	34.2	1,096	3	33.1	2,266
61	6	39.1	4	35.2	1,420	3	34.4	2,180
65	4	38.3	4	34.0	1,471	4	34.1	2,933
69	4	37.5	4	34.3	1,458	4	33.5	2,985
74	4	37.6	4	34.4	1,453	4	33.8	2,959
78	4	38.2	4	35.4	1,412	4	34.3	2,915
82	4	37.5	4	34.8	1,437	4	33.5	2,985
86	4	37.5	4	34.3	1,458	4	33.6	2,976
91	5	38.2	4	34.7	1,441	4	34.0	2,941
95	4	37.5	4	34.2	1,462	4	32.8	3,049
99	4	38.4	4	34.6	1,445	4	33.8	2,959
Mean	4.0	36.1	3.9	32.9	1,492	3.9	32.1	3,030
SD (c)	0.7		0.3		128	0.3		312
CV (d)	17.5		7.7		8.6	7.7		10.3

(a) Grams of feed removed from the feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of tetracycline hydrochloride consumed per day per kilogram of body weight
(c) Standard deviation
(d) Coefficient of variation = (standard deviation/mean) × 100

TABLE G4. FEED AND COMPOUND CONSUMPTION BY FEMALE MICE IN THE TWO-YEAR FEED STUDY OF TETRACYCLINE HYDROCHLORIDE

Week	Control		12,500 ppm			25,000 ppm		
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
3	3	22.4	3	21.0	1,786	3	20.4	3,676
9	3	25.4	3	22.9	1,638	4	22.8	4,386
13	3	28.1	3	24.4	1,537	3	23.6	3,178
17	3	29.6	3	25.3	1,482	3	24.1	3,112
22	4	31.3	3	26.6	1,410	3	25.2	2,976
26	4	33.0	3	26.8	1,399	3	25.4	2,953
31	4	33.2	3	27.3	1,374	4	26.2	3,817
35	3	35.3	3	28.9	1,298	4	26.7	3,745
39	3	36.6	3	29.0	1,293	3	27.1	2,768
43	4	36.4	3	28.3	1,325	3	26.5	2,830
48	4	36.6	4	28.1	1,779	4	26.3	3,802
52	4	35.9	3	28.7	1,307	4	26.6	3,759
57	3	36.2	3	29.7	1,263	3	27.7	2,708
61	4	37.2	4	29.4	1,701	4	27.8	3,597
65	4	37.1	4	30.1	1,661	4	28.2	3,546
69	4	36.5	4	30.2	1,656	4	27.6	3,623
74	4	37.5	4	31.0	1,613	4	27.8	3,597
78	4	37.2	4	30.8	1,623	4	28.2	3,546
82	4	37.2	4	30.9	1,618	4	28.2	3,546
86	4	38.8	4	31.6	1,582	4	29.0	3,448
91	4	39.5	4	31.6	1,582	5	28.6	4,371
95	4	40.0	4	30.4	1,645	4	28.7	3,484
99	4	39.5	4	30.7	1,629	4	29.3	3,413
Mean	3.7	34.8	3.5	28.4	1,530	3.7	26.6	3,473
SD (c)	0.5		0.5		163	0.6		446
CV (d)	13.5		14.3		10.7	16.2		12.8

(a) Grams of feed removed from the feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of tetracycline hydrochloride consumed per day per kilogram of body weight
(c) Standard deviation
(d) Coefficient of variation = (standard deviation/mean) × 100

APPENDIX H

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

Meal Diet: December 1980 to January 1983

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

TABLE H1. 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 H2. 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 H3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION (a)

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Crude protein (percent by weight)	24.25 ± 1.04	22.6-26.3	24
Crude fat (percent by weight)	5.10 ± 0.44	4.4-6.0	24
Crude fiber (percent by weight)	3.38 ± 0.38	2.4-4.2	24
Ash (percent by weight)	6.59 ± 0.34	5.97-7.42	24
Amino Acids (percent of total diet)			
Arginine	1.323 ± 0.830	1.21-1.39	4
Cystine	0.310 ± 0.099	0.218-0.400	4
Glycine	1.155 ± 0.069	1.06-1.21	4
Histidine	0.572 ± 0.030	0.530-0.603	4
Isoleucine	0.910 ± 0.033	0.881-0.944	4
Leucine	1.949 ± 0.065	1.85-1.99	4
Lysine	1.275 ± 0.076	1.20-1.37	4
Methionine	0.422 ± 0.187	0.306-0.699	4
Phenylalanine	0.909 ± 0.167	0.665-1.04	4
Threonine	0.844 ± 0.029	0.824-0.886	4
Tryptophan	0.187	0.171-0.211	3
Tyrosine	0.631 ± 0.094	0.566-0.769	4
Valine	1.11 ± 0.050	1.05-1.17	4
Essential Fatty Acids (percent of total diet)			
Linoleic	2.44	2.37-2.52	3
Linolenic	0.274	2.56-0.308	3
Arachidonic	0.008		1
Vitamins			
Vitamin A (IU/kg)	11,188 ± 1,239	8,900-14,000	24
Vitamin D (IU/kg)	4,650	3,000-6,300	2
α-Tocopherol (ppm)	41.53 ± 7.52	31.1-48.9	4
Thiamine (ppm)	16.2 ± 2.30	12.0-21.0	(b) 23
Riboflavin (ppm)	7.5 ± 0.96	6.1-8.2	4
Niacin (ppm)	85.0 ± 14.2	65.0-97.0	4
Pantothenic acid (ppm)	29.3 ± 4.6	23.0-34.0	4
Pyridoxine (ppm)	7.6 ± 1.5	5.6-8.8	4
Folic acid (ppm)	2.8 ± 0.88	1.8-3.7	4
Biotin (ppm)	0.27 ± 0.05	0.21-0.32	4
Vitamin B_{12} (ppb)	21.0 ± 11.9	11.0-38.0	4
Choline (ppm)	3,302 ± 120.0	3,200.0-3,430.0	4
Minerals			
Calcium (percent)	1.23 ± 0.12	1.10-1.53	24
Phosphorus (percent)	0.97 ± 0.06	0.84-1.10	24
Potassium (percent)	0.862 ± 0.100	0.772-0.974	3
Chloride (percent)	0.546 ± 0.100	0.442-0.635	4
Sodium (percent)	0.311 ± 0.038	0.258-0.350	4
Magnesium (percent)	0.169 ± 0.133	0.151-0.181	4
Sulfur (percent)	0.316 ± 0.070	0.270-0.420	4
Iron (ppm)	447.0 ± 57.3	409.0-523.0	4
Manganese (ppm)	90.6 ± 8.20	81.7-95.5	4
Zinc (ppm)	53.6 ± 5.27	46.1-58.6	4
Copper (ppm)	10.77 ± 3.19	8.09-15.39	4
Iodine (ppm)	2.95 ± 1.05	1.52-3.82	4
Chromium (ppm)	1.81 ± 0.28	1.44-2.09	4
Cobalt (ppm)	0.68 ± 0.14	0.49-0.80	4

(a) One to four batches of feed analyzed for nutrients reported in this table were manufactured during 1983-1985.
(b) One batch (7/22/81) not analyzed for thiamine

TABLE H4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminants	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.44 ± 0.14	0.21-0.93	24
Cadmium (ppm) (a)	<0.1		24
Lead (ppm)	1.03 ± 0.75	0.27-2.93	24
Mercury (ppm) (a)	<0.05		24
Selenium (ppm)	0.27 ± 0.05	0.16-0.40	24
Aflatoxins (ppb) (a,b)	<10	<5.0-10.0	24
Nitrate nitrogen (ppm) (c)	9.35 ± 4.35	0.6-18.0	24
Nitrite nitrogen (ppm) (c)	1.97 ± 1.28	0.4-5.3	24
BHA (ppm) (d)	5.83 ± 5.12	0.4-20.0	24
BHT (ppm) (d)	3.42 ± 2.57	<1-13.0	24
Aerobic plate count (CFU/g) (e)	105,438 ± 75,797	7,000-300,000	24
Coliform (MPN/g) (f)	1,046 ± 973	<3-2,400	24
E. coli (MPN/g) (f,g)	8.0 ± 7.91	<3-23	23
E. coli (MPN/g) (f,h)	13.92 ± 30.00	<3-150	24
Total nitrosamines (ppb) (i, j)	5.13 ± 4.47	<1.2-18.8	22
Total nitrosamines (ppb) (i,k)	13.11 ± 27.39	<1.2-101.6	24
N-Nitrosodimethylamine (ppb) (i,l)	3.82 ± 4.29	0.6-16.8	22
N-Nitrosodimethylamine (ppb) (i,m)	11.71 ± 27.03	0.6-99	24
N-Nitrosopyrrolidine (ppb)	1.21 ± 0.66	<0.3-2.4	24
Pesticides (ppm)			
α-BHC (a,n)	<0.01		24
β-BHC (a,n)	<0.02		24
γ-BHC-Lindane (a,n)	<0.01		24
δ-BHC (a,n)	<0.01		24
Heptachlor (a)	<0.01		24
Aldrin (a)	<0.01		24
Heptachlor epoxide (a)	<0.01		24
DDE (o)	<0.01	0.05 (7/14/81)	24
DDD (a)	<0.01		24
DDT (a)	<0.01		24
HCB (a)	<0.01		24
Mirex (a)	<0.01		24
Methoxychlor (p)	<0.05	0.13 (8/25/81); 0.6 (6/29/82)	24
Dieldrin (a)	<0.01		24
Endrin (a)	<0.01		24
Telodrin (a)	<0.01		24
Chlordane (a)	<0.05		24
Toxaphene (a)	<0.1		24
Estimated PCBs (a)	<0.2		24
Ronnel (a)	<0.01		24
Ethion (a)	<0.02		24
Trithion (a)	<0.05		24
Diazinon (a)	<0.1		24
Methyl parathion (a)	<0.02		24
Ethyl parathion (a)	<0.02		24
Malathion (q)	0.08 ± 0.05	<0.05-0.25	24
Endosulfan I (a)	<0.01		24
Endosulfan II (a)	<0.01		24
Endosulfan sulfate (a)	<0.03		24

TABLE H4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

(a) All values were less than the detection limit, given in the table as the mean.
(b) The detection limit was reduced from 10 ppb to 5 ppb after 7/81.
(c) Sources of contamination: alfalfa, grains, and fish meal
(d) Sources of contamination: soy oil and fish meal
(e) CFU = colony-forming units
(f) MPN = most probable number
(g) Mean, standard deviation, and range exclude one high value of 150 obtained for the batch produced on 8/26/82.
(h) Mean, standard deviation, and range include the high value listed in footnote (g).
(i) All values were corrected for percent recovery.
(j) Mean, standard deviation, and range exclude one high value of 101.6 ppb obtained for the batch produced on 1/26/81 and one high value of 100.3 ppb obtained for the batch produced on 4/27/81.
(k) Mean, standard deviation, and range include the high values listed in footnote (j).
(l) Mean, standard deviation, and range exclude one high value of 97.9 ppb obtained for the batch produced on 1/26/81 and one very high value of 99 ppb obtained for the batch produced on 4/27/81.
(m) Mean, standard deviation, and range include the high values given in footnote (l).
(n) BHC = hexachlorocyclohexane or benzene hexachloride
(o) There was one observation above the detection limit; the value and date it was obtained are given under the range.
(p) There were two observations above the detection limit; the values and dates they were obtained are given under the range.
(q) Eleven batches contained more than 0.05 ppm.

APPENDIX I

AUDIT SUMMARY

APPENDIX I. AUDIT SUMMARY

The experimental data, documents, and pathology specimens for the 2-year studies of tetracycline hydrochloride in rats and mice were audited for accuracy, consistency, completeness, and compliance with the Good Laboratory Practice (GLP) regulations of the Food and Drug Administration (fully implemented by the NTP beginning October 1, 1981). The studies were conducted by Physiological Research Laboratories, Minneapolis, Minnesota. Animal dosing with tetracycline hydrochloride in feed began on February 9, 1981, for rats and February 23, 1981, for mice. The retrospective audit was conducted for the National Institute of Environmental Health Sciences (NIEHS) at the National Toxicology Program (NTP) Archives in April 1987 by Argus Research Laboratories, Inc. The full audit report is on file at the NIEHS. The audit included a review of:

(1) All records concerning animal receipt, quarantine, randomization, and disposition prior to study start.
(2) All inlife records including protocol, correspondence, dosing, environmental conditions, masses, mortality, animal identification, and serology.
(3) Body weight and clinical observation data for a random 10% sample of animals in each dose group.
(4) All chemistry records.
(5) All postmortem records for individual animals concerning disposition codes, condition codes, tissue accountability, correlation of masses or clinical signs recorded at the last inlife observation with gross observations and microscopic diagnoses, and correlations between gross observations and microscopic diagnoses.
(6) All wet tissue bags for inventory, and wet tissues from a random 20% sample of animals from each study group plus other relevant cases to verify animal identity and to examine for untrimmed potential lesions.
(7) Blocks and slides of tissues from a random 20% sample of animals from each dose group to examine for proper match and inventory.
(8) Correlation between original microscopic observations and tabulated pathology diagnoses for a random 10% sample of study animals to verify computer data entry.
(9) The data and results pertaining to the 2-year studies of tetracycline hydrochloride in the draft NTP Technical Report

The audit showed that inlife procedures and events were documented adequately by the study records with the exception of information on disposition of surplus animals and study chemical. A 100% review of masses recorded during the last month of inlife records for each study animal showed that 36/41 masses noted for rats and 27/30 masses noted for mice were correlated with histopathologic observations. The time between death and necropsy exceeded 8 hours for 2 rats and for 14 mice, but tissue accountability was rated good or fair for all target organs. None of these audit findings was considered to affect the interpretation of the studies.

Audit of the pathology specimens for individual animal identifiers showed that punched ears and clipped toes were not saved consistently. Of 72 rats and 67 mice examined, 20 rats and 22 mice were identified by their residual tissues. The remaining animals had either correct but incomplete identifying tissues present (41 rats and 36 mice) or identifying tissues either absent, mutilated, or unreadable (11 rats and 9 mice). None of the animals examined was misidentified by the tissues present; all had correct sex organs present and necropsy observations that were consistent with the residual tissues. Thus, there was no evidence that animals may have been switched. The audit also identified a variety of untrimmed potential lesions (4 in rats, 2 in mice, 1 liver) and gross observations that lacked corresponding microscopic diagnoses (13 in rats, 9 in mice). NTP staff judged these to be relatively minor and not to adversely affect interpretation of the pathology data. Full details about these and other audit findings are presented in the audit report. In conclusion, the study records at the NTP Archives support the data and results presented in the NTP Technical Report.

* U. S. GPO: 1989-244-211