NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF THEOPHYLLINE

(CAS NO. 58-55-9)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED AND GAVAGE STUDIES)

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

August 1998

NTP TR 473

NIH Publication No. 98-3963

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

FOREWORD

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

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

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

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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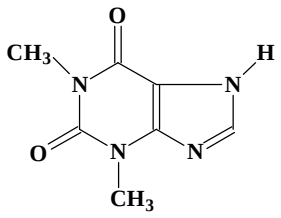
ABSTRACT



THEOPHYLLINE

CAS No. 58-55-9

Chemical Formula: $C_7H_8N_4O_2$ Molecular Weight: 180.17

Synonyms: 3,7-dihydro-1,3-dimethyl-1H-purine-2,6-dione; 1,3-dimethylxanthine; 1H-purine-2,6-dione; NSC 2066; pseudotheophylline; theocin; theophylline, anhydrous

Trade names: Accurbron; Aerobin; Aerolate III; Afonilum; Aminophylline; Aquaphyllin; Armophylline; Asmalix; Bilordyl; Bronchoretard; Bronkodyl; Cetraphylline; Constant-T; Diffumal; Duraphyl; Duraphyllin; Elixicon; Elixophyllin; Euphylline L.A.; Euphylong; LaBID; Labophylline; Lanophyllin; Lasma; Liquophylline; Optiphyllin; Parkophyllin; Phylocontin; Physpan; Pro-Vent; PulmiDur; Pulmo-Timelets; Quibron; Respbid; Rona-Phyllin; Sabidal; Slo-bid; Slo-Phyllin; Solosin; Sustaire; Tefamin; Teobid; Teofyllamin; Tesona; Theal tablets; Theo-24; Theobid; Theocap; Theochron; Theoclear; Theocontin; Theo-Dur; Theofol; Theograd; Theolair; Theolan; Theolix; Theophyl; Theoplus; Theo-Sav; Theosol; Theospan; Theostat; Theovent; TheoX; T-Phyl; Truphylline; Uni-Dur; Unifyl; Uniphyl; Uniphyllin; Xanthium

Theophylline is an alkaloid found in tea (*Thea sinensis*) and chocolate and is structurally related to caffeine and theobromine. Theophylline is used as a pharmaceutical agent. It stimulates the heart and central nervous system, relaxes the smooth muscles of the bronchi and blood vessels, and causes diuresis. The drug is used mainly as a bronchodilator in obstructive airway diseases, such as bronchial asthma, and for myocardial stimulation. Theophylline was nominated for toxicologic and carcinogenicity testing as a representative of the purine structural subclass, particularly because of its relationship to purines such as caffeine, 1-methyl-3-hydroxyguanine, and 3-hydroxy-1-methylxanthine, the latter two compounds having been shown to induce sarcomas in rats. Additional reasons for testing theophylline included its widespread use in humans as a pharmaceutical agent, its possible genotoxicity *in vitro*, and the lack of information on its potential toxicity and/or carcinogenicity under conditions of chronic oral usage. Based on reported teratogenicity and testicular toxicity, it was also recommended that reproductive studies be included in the evaluation of theophylline. The oral route of administration was selected because it is the primary route of human exposure, and the gavage route was selected because it mimics the pharmaceutical use of theophylline in humans. Male and female F344/N rats and $B6C3F_1$ mice were given theophylline (greater than 99% pure) in feed or in corn oil by gavage for 16 days or 14 weeks or in corn oil by gavage for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, mouse bone marrow, and mouse peripheral blood.

16-DAY FEED STUDY IN RATS

Groups of five male and five female F344/N rats were given 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm theophylline in feed for 16 days, which resulted in approximate daily doses of 50, 100, 250, 450, or 1,000 mg theophylline/kg body weight to males and 75, 150, 250, 450, or 1,100 mg/kg to females. All rats

survived until the end of the study. The final mean body weights and body weight gains of 8,000 ppm males and females were significantly less than those of the controls. The absolute and relative testis weights of 4,000 ppm males were significantly greater than those of the controls. Increased incidences of uterine hypoplasia were observed microscopically in exposed groups of females.

16-DAY GAVAGE STUDY IN RATS

Groups of five male and five female F344/N rats were given 0, 12.5 (twice daily), 25 (once daily), 50 (once daily), 50 (twice daily), 100 (once daily), 200 (once daily), 200 (twice daily), or 400 (once daily) mg theophylline/kg body weight in corn oil by gavage. All rats receiving 400 mg/kg once daily and all but one female receiving 200 mg/kg twice daily died during the study. In groups dosed once daily, final mean body weights and body weight gains of males receiving 100 or 200 mg/kg and mean body weight gains of females receiving 50, 100, or 200 mg/kg were less than those of controls. The final mean body weights and body weight gains of groups receiving theophylline twice daily were generally similar to those of groups receiving the same daily dosages once daily. Clinical findings included rapid or labored respiration, hunched posture, and squinting. In groups dosed once daily, absolute and relative uterus weights of females receiving 100 or 200 mg/kg once daily were significantly less than those of the controls, and the absolute and relative uterus weights of females receiving 100 mg/kg once daily were significantly less than those of females receiving 50 mg/kg twice daily. Uterine atrophy was observed in three females receiving 200 mg/kg twice daily. Periarteritis of the mesenteric arteries was observed in two males and two females receiving 400 mg/kg once daily.

16-DAY FEED STUDY IN MICE

Groups of five male and five female $B6C3F_1$ mice were given 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm theophylline in feed for 16 days, resulting in approximate daily doses of 250, 475, 950, 1,800, or 2,000 mg theophylline/kg body weight to males and 300, 450, 1,225, 2,000, or 4,375 mg/kg to females. All mice survived until the end of the study. Final mean body weights of 4,000 and 8,000 ppm females and mean body weight gains of 2,000, 4,000, and 8,000 ppm females were significantly greater than those of the controls. Feed consumption by exposed groups was similar to that by the controls, except that by the 8,000 ppm males, which was approximately 40% the amount of feed consumed by the control group. Histopathologic examinations were not performed due to the absence of mortality and significant exposure-related lesions.

16-DAY GAVAGE STUDY IN MICE

Groups of five male and five female $B6C3F_1$ mice were given 0, 12.5 (twice daily), 25 (once daily), 50 (once daily), 50 (twice daily), 100 (once daily), 200 (once daily), 200 (twice daily), or 400 (once daily) mg theophylline/kg body weight in corn oil by gavage. Three males and all females receiving 400 mg/kg once daily died on day 1. There were no significant differences in final mean body weights or body weight gains. There were no histopathologic findings attributed directly to theophylline.

14-WEEK FEED STUDY IN RATS

Groups of 10 male and 10 female F344/N rats were given 0, 1,000, 2,000, or 4,000 ppm theophylline in feed for 14 weeks, which resulted in approximate daily doses of 75, 125, or 250 mg theophylline/kg body weight to males and 75, 125, or 275 mg/kg to females. The final mean body weight of 1,000 ppm females was significantly greater than that of the control group. Feed consumption by exposed groups was similar to that by the controls. Mean cell volume and mean cell hemoglobin were significantly greater in males exposed to 2,000 or 4,000 ppm than those in the control group. Segmented neutrophil counts of all groups of exposed females were significantly greater than that of the control group. The absolute and relative kidney weights of 4,000 ppm males were significantly greater than those of the controls, and there was an exposure-related increase in the severity of nephropathy in males. Exposure-related increases in the incidences of mesenteric and/or pancreatic periarteritis were observed in males and females.

14-WEEK GAVAGE STUDY IN RATS

Groups of 10 male and 10 female F344/N rats were given 0, 37.5, 75, or 150 mg theophylline/kg body weight in corn oil by gavage for 14 weeks. One male and one female receiving 150 mg/kg died before the end of the study. The mean body weight gain of 150 mg/kg females was significantly greater than that of the controls. Mean cell volume of 150 mg/kg males and mean cell hemoglobin of all groups of dosed males were significantly greater than those of the control group. There were slight dose-dependent increases in the incidences of mesenteric periarteritis in dosed males and females.

14-WEEK FEED STUDY IN MICE

Groups of 10 male and 10 female $B6C3F_1$ mice were given 0, 1,000, 2,000, or 4,000 ppm theophylline in feed for 14 weeks, resulting in approximate daily doses of 175, 400, or 800 mg theophylline/kg body weight to males and 225, 425, or 850 mg/kg to females. All mice survived until the end of the study. The final mean body weights and body weight gains of all exposed groups of males and females were significantly less than those of the controls. Feed consumption by exposed groups was similar to that by the controls. Leukocyte, segmented neutrophil, and lymphocyte counts of 4,000 ppm males were significantly greater than those of the controls. Leukocyte and segmented neutrophil counts of 2,000 or 4,000 ppm females were significantly greater than those of the controls. There were no histopathologic findings attributed directly to theophylline exposure.

14-WEEK GAVAGE STUDY IN MICE

Groups of 10 male and 10 female $B6C3F_1$ mice were given 0, 75, 150, or 300 mg theophylline/kg body weight in corn oil by gavage for 14 weeks. Three males and all females receiving 300 mg/kg, one 75 mg/kg male, and one control female died before the end of the study. Final mean body weights and body weight gains of 150 and 300 mg/kg males were significantly less than those of the controls. Mean cell volume and mean cell hemoglobin of 300 mg/kg males were significantly greater than those of the controls. There were no histopathologic findings attributed directly to theophylline treatment.

2-YEAR GAVAGE STUDY IN RATS

Groups of 50 male and 50 female rats were given 7.5, 25, or 75 mg theophylline/kg body weight in corn oil by gavage for 2 years.

Survival and Body Weights

There were no significant differences in survival between dosed and control groups. Final mean body weights of all groups of dosed males and females were significantly less than those of the controls.

Pathology Findings

There were no significantly increased incidences of neoplasms in dosed rats. The incidence of chronic inflammation of the mesenteric arteries was significantly increased in males receiving 75 mg/kg compared to the controls. There were dose-related negative trends in the incidences of mammary gland fibroadenoma and fibroadenoma or carcinoma (combined) in females; these differences correlated with decreased body weights.

2-YEAR GAVAGE STUDY IN MICE

Groups of 50 male $B6C3F_1$ mice were given 0, 15, 50, or 150 mg theophylline/kg body weight and groups of 50 female $B6C3F_1$ mice were given 0, 7.5, 25, or 75 mg/kg in corn oil by gavage for 2 years.

Survival and Body Weights

Survival of 150 mg/kg males was significantly less than that of the controls. The final mean body weights of 150 mg/kg males, 25 mg/kg females, and 75 mg/kg females were significantly less than those of the control groups.

Pathology Findings

There were no treatment-related increases in incidences of nonneoplastic lesions or neoplasms. In males and females, there were decreased incidences of hepatocellular adenoma and of the combined incidences of hepatocellular adenoma or carcinoma compared to the controls. Male mice had a pattern of nonneoplastic liver lesions along with silver-staining helical organisms in the liver consistent with *Helicobacter hepaticus* infection. The incidences of these liver lesions in 150 mg/kg males were significantly lower than those in control males. Increases in the incidences of hepatocellular neoplasms in male

mice have been shown to be associated with *H. hepaticus* infection when hepatitis is also present. Because of this association, interpretation of the decreased incidence of liver neoplasms in male mice was more difficult. Incidences of lesions at other sites in this study were not considered to have been significantly impacted by *H. hepaticus* infection or its associated hepatitis.

GENETIC TOXICOLOGY

Theophylline was not mutagenic in *Salmonella typhimurium*, with or without metabolic activation (S9). It induced sister chromatid exchanges but not chromosomal aberrations in cultured Chinese hamster ovary cells. The positive sister chromatid exchange response was noted only in the absence of S9. *In vivo*, a mouse bone marrow sister chromatid exchange test showed positive results at a standard 23-hour harvest time; however, this test was not repeated and the response is unconfirmed. An *in vivo* mouse bone marrow chromosomal aberrations test, that employed both standard and extended exposure protocols, gave negative results. The frequency of micronucleated erythrocytes was determined in peripheral blood of male and female mice exposed to theophylline in dosed feed or in corn oil by gavage for 14 weeks. No significant increases in the frequencies of micronucleated cells were seen in male or female mice in either of the studies.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of theophylline in male or female F344/N rats administered 7.5, 25, or 75 mg/kg. There was *no evidence of carcinogenic activity* of theophylline in male B6C3F_1 mice administered 15, 50, or 150 mg/kg or female B6C3F_1 mice administered 7.5, 25, or 75 mg/kg.

Gavage administration of theophylline caused chronic inflammation of the mesenteric arteries in dosed male rats.

Decreased incidences of mammary neoplasms in female rats were likely associated with lower body weights. There were dose-related decreases in the incidences of hepatocellular adenoma and hepatocellular carcinoma in male and female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

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

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 7.5, 25, or 75 mg/kg in corn oil by gavage	0, 7.5, 25, or 75 mg/kg in corn oil by gavage	0, 15, 50, or 150 mg/kg in corn oil by gavage	0, 7.5, 25, or 75 mg/kg in corn oil by gavage
Body weights	Dosed groups less than control group	Dosed groups less than control group	150 mg/kg group less control group	25 and 75 mg/kg groups less than control group
2-Year survival rates	23/50, 33/50, 29/50, 24/50	32/50, 30/50, 33/50, 33/50	36/50, 35/50, 44/50, 26/50	37/50, 37/50, 34/50, 33/50
Nonneoplastic effects	Mesenteric artery: chronic inflammation (2/50, 2/50, 3/50, 15/50)	None	None	None
Neoplastic effects	None	None	None	None
Decreased incidences	None	Mammary gland: fibroadenoma (22/50, 19/50, 12/50, 12/50); fibroadenoma or carcinoma (23/50, 20/50, 12/50, 12/50)	Liver: hepatocellular adenoma (21/50, 18/50, 12/50, 2/50); hepatocellular carcinoma (19/50, 14/50, 12/50, 2/50); hepatocellular adenoma or carcinoma (34/50, 27/50, 22/50, 4/50)	Liver: hepatocellular adenoma (20/50, 11/50, 12/50, 3/50); hepatocellular carcinoma (11/50, 5/50, 6/50, 5/50); hepatocellular adenoma or carcinoma (29/50, 14/50, 18/50, 8/50)
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence

Genetic toxicology

Salmonella typhimurium gene mutations:	Negative in strains TA97, TA98, TA100, and TA1535
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro*:	Positive without S9
Mouse bone marrow *in vivo*:	Positive
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with or without S9
Mouse bone marrow *in vivo*:	Negative
Micronucleated erythrocytes	
Mouse peripheral blood *in vivo* (feed study):	Negative
Mouse peripheral blood *in vivo* (gavage study):	Negative

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on theophylline on 11 December 1996 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 11 December 1996 the draft Technical Report on the toxicology and carcinogenicity studies of theophylline received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. P.C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of theophylline by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related nonneoplastic lesions in male rats. The proposed conclusions for the 2-year studies in rats and mice were *no evidence of carcinogenic activity* in male or female F344/N rats or B6C3F$_1$ mice.

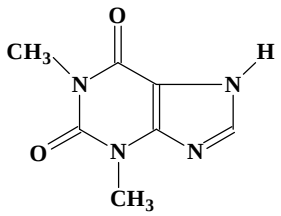
Dr. Reddy, a principal reviewer, agreed with the proposed conclusions. He said it would be useful to include information on the theophylline concentration per cup of tea and average daily consumption in tea drinkers. Dr. Chan said that it was a very small amount but wide ranging due to different kinds of tea and preparations. Dr. Reddy asked why the decision was made not to do histopathologic examination of tissues from mice fed theophylline for 16 days. Dr. Chan said that these animals were used only for dose selection and there was no mortality. Dr. Reddy wondered whether the periarteritis was due to the drug or to the *Helicobacter* infection. Dr. J.R. Hailey, NIEHS, observed that *Helicobacter* is not reported to have effects on vasculature outside of the liver.

Dr. Taylor, the second principal reviewer, agreed with the proposed conclusions. He thought a more extensive discussion of the periarteritis should be included, noting that in human medicine this can represent a fairly serious and life-threatening condition, which can occur after the administration of certain drugs. Dr. A. Nyska, NIEHS, commented that this lesion is characteristic of vasodilator drugs and was observed only in rats and only in the mesenteric arteries.

Dr. W.T. Allaben, NCTR/FDA, recommended that comments be made in the conclusions regarding the decreases in liver cancer in treated mice and mammary gland cancer in rats. Dr. J.R. Bucher, NIEHS, said this would be done. Dr. Goldsworthy said there also should be comment on significant decreases in body weight gain in the conclusions.

Dr. Reddy moved that the Technical Report on theophylline be accepted with the revisions discussed and the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity*. Dr. Taylor seconded the motion, which was accepted unanimously with nine votes.

INTRODUCTION



THEOPHYLLINE

CAS No. 58-55-9

Chemical Formula: $C_7H_8N_4O_2$ Molecular Weight: 180.17

Synonyms: 3,7-dihydro-1,3-dimethyl-1H-purine-2,6-dione; 1,3-dimethylxanthine; 1H-purine-2,6-dione; NSC 2066; pseudotheophylline; theocin; theophylline, anhydrous

Trade names: Accurbron; Aerobin; Aerolate III; Afonilum; Aminophylline; Aquaphyllin; Armophylline; Asmalix; Bilordyl; Bronchoretard; Bronkodyl; Cetraphylline; Constant-T; Diffumal; Duraphyl; Duraphyllin; Elixicon; Elixophyllin; Euphylline L.A.; Euphylong; LaBID; Labophylline; Lanophyllin; Lasma; Liquophylline; Optiphyllin; Parkophyllin; Phylocontin; Physpan; Pro-Vent; PulmiDur; Pulmo-Timelets; Quibron; Respbid; Rona-Phyllin; Sabidal; Slo-bid; Slo-Phyllin; Solosin; Sustaire; Tefamin; Teobid; Teofyllamin; Tesona; Theal tablets; Theo-24; Theobid; Theocap; Theochron; Theoclear; Theocontin; Theo-Dur; Theofol; Theograd; Theolair; Theolan; Theolix; Theophyl; Theoplus; Theo-Sav; Theosol; Theospan; Theostat; Theovent; TheoX; T-Phyl; Truphylline; Uni-Dur; Unifyl; Uniphyl; Uniphyllin; Xanthium

CHEMICAL AND PHYSICAL PROPERTIES

Theophylline is a bitter tasting, odorless, white crystalline powder with a melting point of 271° to 274° C. Theophylline is moderately soluble in water (1 g/120 mL), alcohol (1 g/80 mL), chloroform (1 g/110 mL), alkali hydroxides, ammonia, diluted hydrochloric acid, and nitric acid and is slightly soluble in ether (*Merck Index*, 1989; *Hazardous Chemicals Desk Reference*, 1993).

PRODUCTION, USE, AND HUMAN EXPOSURE

Theophylline is an alkaloid found in tea (*Thea sinensis*) and chocolate and is structurally related to caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethyxanthine). Theophylline is used as a pharmaceutical agent. It stimulates the heart and central nervous system, relaxes smooth muscles of the bronchi and blood vessels, and causes diuresis. Theophylline is used mainly as a bronchodilator in obstructive airway diseases, such as bronchial asthma, and for myocardial stimulation. The therapeutic doses of theophylline range from 3 to 6 mg/kg, yielding a serum level of 10 to 20 µg/mL (Kodama *et al.*, 1980). Theophylline is an inhibitor of tumor necrosis factor alpha (TNF-α) (Semmler *et al.*, 1993) and therefore has therapeutic use in the treatment of chronic obstructive pulmonary disease, a disease in which TNF-α has been shown to play a pathogenic role (Semmler *et al.*, 1993; Di Francia *et al.*, 1994).

MECHANISMS OF ACTION

Theophylline is a methylxanthine structurally similar to purines and purine bases (Cornish and Christman, 1957; Grygiel and Birkett, 1980). Many of theophylline's actions are related to competition with adenosine for adenosine receptors (Bruns *et al.*,

1980; *Goodman and Gilman's*, 1990). Theophylline inhibits the guanine nucleotide-binding protein G_i (Schrader *et al.*, 1987). Theophylline reduces the immunological release of histamine (Berti *et al.*, 1990).

Theophylline competitively inhibits cyclic nucleotide phosphodiesterase activity (Berardi *et al.*, 1996). Through this interference, theophylline inhibits the enzyme that catalyzes the breakdown of the intracellular messenger cyclic 3′,5′-adenylic acid (cAMP) to 5′-adenylic acid (AMP). The accumulation of cAMP increases the actions of neurotransmitters and hormones, such as catecholamine, that are mediated by intracellular cAMP. Theophylline also inhibits certain purine nucleoside phosphorylases.

High levels of theophylline trigger release of norepinephrine, causing an increase in the number of slow Ca^{2+} channels available for voltage activation through which Ca^{2+} can pass during the action potential. This mobilization of Ca^{2+} affects skeletal muscle and neuromuscular synaptic transmission and stimulates the release of catecholamine from the adrenal medulla. Increased intracellular Ca^{2+} can cause electrolyte changes, cardiac arrhythmias, hypotension, and gastrointestinal disturbances (Sperelakis, 1992). The combination of elevated cAMP and potentiation of catecholamine and other hormones causes relaxation of smooth muscle, most notably of the bronchi and blood vessels. These factors as well as direct effects of calcium on the contractile apparatus of the heart are responsible for the cardiac effects of theophylline.

In vitro, theophylline inhibited beef heart cGMP phosphodiesterase activity; the inhibitory effect was dose dependent. The IC_{50} (concentration giving 50% inhibition) was 2.91 ± 0.41 mM at 50 µM cyclic GMP (Berardi *et al.*, 1996).

Theophylline can be incorporated into DNA (Steinberg and Whittaker, 1978) and reportedly interferes with normal DNA synthesis, mitosis, and post-replication DNA repair (Timson, 1972; Bender *et al.*, 1974; Weinstein *et al.*, 1975; Murnane *et al.*, 1981). Theophylline inhibited cloning efficiency and cellular growth rate of cultured Chinese hamster ovary cells. It also induced sister chromatid exchanges and potentiated the toxic effects of methylnitrosourea in Chinese hamster ovary cells (Morris and Heflich, 1984). Theophylline exerts its effects probably by inhibiting DNA synthesis, mitosis, and post-replication DNA repair (Timson 1972; Weinstein *et al.*, 1975; Zajdela and Latarjet, 1978; Murnane *et al.*, 1981; Morris and Heflich, 1984).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

In male and female Sprague-Dawley rats, 8-[^{14}C]-theophylline was metabolized to 1,3-dimethyluric acid and 1-methyluric acid. No 3-methylxanthine was found. The biological half-life, determined from urinary excretion of radioactivity, was 6 ± 1.5 hours. The half-life of theophylline in the blood of male Sprague-Dawley rats is 3.5 hours (Williams *et al.*, 1979). Theophylline appears to be metabolized solely by liver microsomal P_{450} enzymes; evidence of metabolism was not found in the heart, lung, intestine, brain, adrenal glands, kidney, or spleen (Lohmann and Miech, 1976).

Induction of hepatic drug-metabolizing activity increases the rate of theophylline metabolism. The rate of theophylline metabolism therefore depends on a combination of genetic factors and exposure to inducers or inhibitors of the hepatic drug-metabolizing enzyme system. Williams *et al*. (1979) demonstrated that inducers such as phenobarbital and 3-methylcholanthrene significantly lower theophylline plasma half-life values in Sprague-Dawley rats. On the other hand, naringenin, a derivative of naringin from grapefruit, is a potent inhibitor of human cytochrome P_{450} isoforms and prolongs the plasma half-life of caffeine; however, the plasma half-life of theophylline was not prolonged by exposure to grapefruit juice (Furh *et al.*, 1995). Pretreatment with theophylline or 3-methylcholanthrene resulted in a faster rate of metabolism of theophylline in Sprague-Dawley rats, indicating induction of metabolic enzymes (Lohmann and Miech, 1976).

Humans

Theophylline is readily absorbed after oral administration (approximately 96% of an uncoated theophylline tablet is absorbed) and maximal blood concentrations are reached within 30 to 120 minutes

(Ogilvie, 1978). It is absorbed slowly after intramuscular administration (Mitenko and Ogilvie, 1973; Ogilvie, 1978).

Müller *et al.* (1995) examined theophylline concentrations in plasma, muscle, and adipose tissue in men administered 300 mg orally or 240 mg intravenously. Microdialysis probes, inserted into the medial vastus muscle and the periumbilical subcutaneous adipose layer, were used to measure concentrations in muscle and adipose tissue. Müller *et al.* (1995) reported maximum plasma concentrations at 56 minutes following oral administration or 20 minutes following intravenous administration. Maximum plasma concentrations were 6.1 µg/mL (oral administration) or 8.3 µg/mL (intravenous administration). Using area-under-the-curve (AUC) ratios to determine the relative concentrations of theophylline in tissue compared to plasma, the $AUC_{tissue}/AUC_{plasma}$ ratios for muscle were 0.56 (oral administration) and 0.55 (intravenous administration), and the mean $AUC_{tissue}/AUC_{plasma}$ ratios for adipose tissue were 0.55 (oral adminis-tration) and 0.72 (intravenous administration).

Theophylline is reversibly bound to plasma proteins and distributed in erythrocytes, saliva, breast milk, and amniotic fluid, and can cross the placenta (Ogilvie, 1978). The drug accumulates in the fetus and is eliminated slowly (Arwood *et al.*, 1979). Within a concentration range of 10 to 20 µg/mL, theophylline is bound (55%) to protein *in vivo*. Certain drugs displace theophylline from protein binding.

Within the therapeutic range, theophylline is metabolized by first-order kinetics (Minton and Henry, 1996); however, at high concentrations metabolic enzymes become saturated and zero-order kinetics become evident (*Goodman and Gilman's*, 1990). Figure 1 shows the primary pathways of theophylline metabolism. In humans, theophylline is metabolized by the liver microsomal mixed-function oxidase system using more than one cytochrome P_{450} isoform (Minton and Henry, 1996). CYP1A2 is the major isoform and is responsible for N-demethylation and 8-hydroxylation. Principal substrates for CYP1A2 are phenacetin, caffeine, theophylline, and testosterone. CYP2E1, a low-affinity but high-capacity isoform, plays a minor role through hydroxylation. Theophylline is metabolized to 1,3-dimethyluric acid, 3-methylxanthine, or 1-methylxanthine, which is rapidly converted to 1-methyluric acid by xanthine oxidase. These metabolites are then excreted without further alteration. After administration of a 1-gram oral dose to each of two human volunteers, the following percentages of metabolites were found in the urine: 1,3-dimethyluric acid, 35%; 1-methyluric acid, 19%; 3-methylxanthine, 13%; and unchanged theophylline, 10% (Cornish and Christman, 1957). 1-Methylxanthine and 3-methyluric acid have also been found in human urine (Grygiel and Birkett, 1980). In adult liver, theophylline is formed during N7-demethylation of caffeine, with 4% of caffeine being converted to theophylline (Tassaneeyakul *et al.*, 1994), but theophylline is not converted to caffeine (Aranda *et al.*, 1979).

Because theophylline is metabolized by liver P_{450} enzymes, metabolism is subject to individual genetic variations, disease state, and age. There is little correlation between toxic effects and dosage because of a high degree of variability between individuals in the relationship between dosage and blood concentrations of theophylline (Jacobs *et al.*, 1976). Jacobs *et al.* (1976) examined blood levels in eight healthy volunteers and found peak serum concentrations were similar but found wide variation in elimination half-life, which ranged from 4 to 10 hours. Over the course of a therapeutic regimen, this could result in widely varying plasma concentrations.

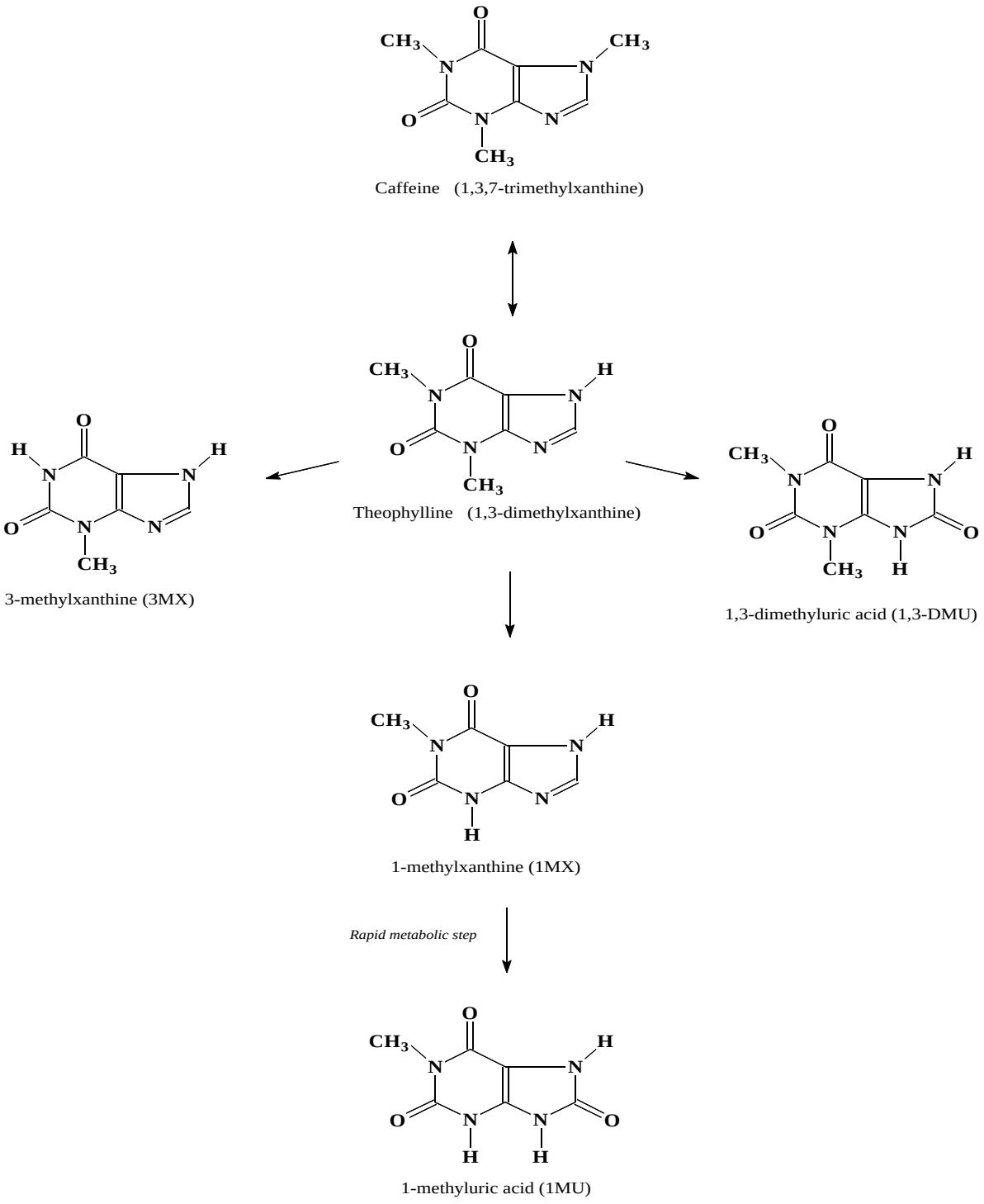


FIGURE 1
Pathways of Theophylline Metabolism (Minton and Henry, 1996)

The plasma half-life in children is about half that of adults (Grygiel and Birkett, 1980), while the half-life of theophylline is considerably longer in newborns (approximately 30 hours in newborns and 6 hours in adults) (Aranda *et al.*, 1979). Indications are that metabolic pathways active in children and adults are minimally functional in fetuses or preterm neonates (Aranda *et al.*, 1979; Grygiel and Birkett, 1980) and that metabolism of theophylline in fetuses primarily involves methylation to caffeine (Aranda *et al.*, 1979). Grygiel and Birkett (1980) compared plasma levels and urine metabolites in preterm neonates, children, and adults. They found plasma concentrations (corrected for equal dosing) in the neonates to be three times that of adults. Over the length of a dosing interval, 98% of the theophylline administered to the neonates was excreted as unchanged theophylline and the remaining 2% was excreted as caffeine. Aranda *et al*. (1979) examined metabolism of theophylline in liver explants obtained from human fetuses at gestational ages ranging from 12 to 20 weeks. After a 4- to 8-hour lag phase, they found caffeine production over 52 hours was linear at a rate of 1.25 nmol caffeine per mg protein every 24 hours. The amount of caffeine produced was five times the combined amount of 1,3-dimethyluric acid and 3-methylxanthine produced.

Other drugs can influence theophylline clearance (Minton and Henry, 1996). Drugs that reduce clearance include the antidepressants viloxazine (a noradrenaline reuptake inhibitor) and fluvoxamine (a selective serotonin-reuptake inhibitor); the calcium antagonists nifedipine, verapamil, and diltiazem; the H_2-receptor antagonists cimetidine and famotidine; the oral contraceptive pill; and many antibiotics, including erythromycin, ciprofloxacin, and allopurinol. Phenytoin, phenobarbitone, mexiletine, tobacco smoking, and marijuana smoking increase clearance.

TOXICITY

Experimental Animals

For rats, the LD_{50} for oral administration was between 100 and 325 mg/kg body weight and for intraperitoneal administration was 188 mg/kg. For mice, the LD_{50} for oral administration was between 150 and 600 mg/kg, for intraperitoneal administration was 200 mg/kg, and for subcutaneous administration was 184 mg/kg (RTECS, 1982).

Whitehurst *et al*. (1996) reported that intraperitoneal administration of 150 mg/kg theophylline to male and female Sprague-Dawley rats induced myocardial lesions in the left and right ventricular free wall, the papillary muscles, and the septum of the left ventricle. The lesions consisted of small foci or multiple areas of muscle necrosis associated with interstitial edema and inflammatory cell infiltration. Within 20 to 25 minutes after injection, 90% of the rats died.

A high incidence of testicular atrophy was observed in Holtzman rats fed a diet containing 0.5% theophylline for 19 weeks (Friedman *et al.*, 1979).

In male Wistar and male Sprague-Dawley rats, a diet containing 1.39 g/kg theophylline increased calcium excretion by greater than 300% that of controls (Whiting and Whitney, 1987).

Humans

Theophylline has a low therapeutic index (Ogilvie, 1978). The accepted therapeutic serum concentration of theophylline ranges from 10 to 20 µg/mL (Minton and Henry, 1996) and signs of mild toxicity (nausea and vomiting) are seen at 15 µg/mL (Jacobs *et al.*, 1976; Minton and Henry, 1996). In a study of 47 hospitalized individuals, Jacobs *et al*. (1976) found a strong correlation between blood concentration and toxic effects. Toxic symptoms were common at serum concentrations over 25 µg/mL but were not seen at serum concentrations below 15 µg/mL. The most common symptoms were gastrointestinal (nausea, vomiting, or diarrhea) and occurred at 15 µg/mL. One patient experienced agitation (28.5 µg/mL), one patient experienced tremors (26.4 µg/mL), one patient experienced seizure (39.9 µg/mL), and two experienced tachycardia (46.3 µg/mL and 49.5 µg/mL).

Clinical features of theophylline toxicity include metabolic disturbances (hypokalemia, hyperglycemia, hypomagnesemia, metabolic acidosis, and respiratory alkalosis) and effects on the gastrointestinal system (nausea and vomiting), cardiovascular system (tachydysrhythmias), and central nervous system (agitation, tremor, and focal or generalized convulsions or seizures) (Yasuhara and Levy, 1988; Minton and Henry, 1996).

Death from theophylline intoxication is more common than from caffeine intoxication. Rapid intravenous administration of therapeutic doses of 500 mg aminophylline have resulted in death. Most toxicity is associated with long-term oral or parenteral exposure. Although seizures are rare at plasma concentrations below 40 µg/mL, convulsions and death have occurred at concentrations as low as 25 µg/mL (*Goodman and Gilman's*, 1990).

Theophylline increases the urinary output of magnesium, calcium, and sodium and decreases serum levels of phosphate (Knutsen *et al.*, 1994). The long-term effects of altering calcium metabolism are not known.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Theophylline may be a testicular toxicant. Feeding 0.5% theophylline to rats for 14 to 75 weeks resulted in bilateral testicular atrophy with variable atrophic changes in the epididymis, prostate gland, and seminal vesicles (Weinberger *et al.*, 1978; Friedman *et al.*, 1979). In continuous breeding studies, male Swiss (CD-1®) mice exposed to theophylline had reduced seminal vesicle weights and cauda epididymal sperm counts (NTP, unpublished report). Dose-related decreases in gravid uterine weight were observed in CD rats and CD-1 mice given up to 0.4% or 0.2% theophylline, respectively, in drinking water during gestation days 6 through 15 (George *et al.*, 1986).

In CD rats administered up to 0.4% theophylline in drinking water during gestation days 6 through 15, live litter size and fetal weight were reduced, but there was no increase in the incidence of malformations (George *et al.*, 1986). Theophylline administered on gestation days 6 through 15 to Sprague-Dawley rats at up to 0.4% in feed and to CD-1 mice at up to 0.2% in drinking water induced increases in the percentage of resorptions per litter, reduced the number of live fetuses per litter, and decreased average fetal weight per litter (Lindström *et al.*, 1990).

Theophylline is a reported teratogen in mice. In CD-1 mice administered up to 0.2% theophylline in drinking water during gestation days 6 through 15, the percentage of resorptions per litter was increased, average fetal weight was decreased, and there were dose-related increasing trends in the percentage of litters with malformed fetuses and the percentage of malformed fetuses per litter (George *et al.*, 1986). Theophylline administered intraperitoneally at up to 225 mg/kg on day 12 of gestation produced digital defects, cleft palate, micrognathia, and hematomas in the fetuses of ICR-JCL mice (Fujii and Nishimura, 1969). Single intraperitoneal doses of up to 200 mg/kg administered on gestation days 10, 11, 12, or 13 produced cleft palates, limb anomalies (ectrodactyly, syndactyly, micromelia, polydactyly), and embryolethality in ICR mice (Tucci and Skalko, 1978).

Humans

No information related to the reproductive and developmental toxicity of theophylline in humans has been reported in the literature.

CARCINOGENICITY

Experimental Animals

Theophylline has been reported to inhibit the development of skin neoplasms induced by ultraviolet light (Zajdela and Latarjet, 1978), possibly reflecting the ability of theophylline to inhibit error-prone post-replication DNA repair. Theophylline and caffeine (to a greater extent) interfere with the transformation of epidermal cells in culture by dimethylbenz(a)-anthracene by inhibiting the binding of dimethylbenz(a)anthracene to cellular DNA (Shoyab, 1979). Theophylline partially suppresses neoplasm production (Reddi and Constantinides, 1972).

Humans

No information related to the carcinogenicity of theophylline in humans has been reported in the literature.

GENETIC TOXICITY

Theophylline was not mutagenic in *Salmonella typhimurium*, with or without induced liver S9 metabolic activation enzymes (Zeiger *et al.*, 1988), but it was reported to be positive in mutagenicity tests with *Escherichia coli* (Timson, 1975). Theophylline has been shown to induce chromosomal damage in mammalian cells *in vitro*. It was reported to induce sister

chromatid exchanges in cultured Chinese hamster ovary cells and human lymphocytes (Kawachi *et al.*, 1980; Morris and Heflich, 1984; Day *et al.*, 1989) and chromosomal aberrations in human lymphocytes (Weinstein *et al.*, 1975; Day *et al.*, 1989) and various mouse cell lines (Kodama *et al.*, 1980). The chromosomal effects noted by Day *et al.* (1989) in human lymphocytes occurred at concentrations equal to or greater than 10 µg/mL, a concentration that corresponds to *in vivo* serum levels (10-20 µg/mL) attained during therapeutic administration of theophylline to humans. Results of an *in vivo* mouse bone marrow study were positive for induction of sister chromatid exchanges and negative for chromosomal aberrations following a single intraperitoneal injection of up to 250 mg/kg theophylline (McFee, 1991). Elevated sister chromatid exchange levels were also reported in bone marrow cells of Chinese hamsters administered theophylline (doses up to 600 mg/kg) by gavage (Renner, 1982).

STUDY RATIONALE

Theophylline was nominated by the National Cancer Institute for toxicologic and carcinogenicity testing as part of a class study on alkaloid compounds. It was selected for study as a representative of the purine structural subclass, particularly because of its relationship to purines such as caffeine, 1-methyl-3-hydroxyguanine, and 3-hydroxy-1-methyl-xanthine. The latter two compounds have been shown to induce sarcomas in rats when injected subcutaneously (Clayson and Garner, 1976). Additional reasons for selecting theophylline for testing included its widespread use in humans as a pharmaceutical agent, its possible genotoxicity *in vitro*, and the lack of information on its potential toxicity and/or carcinogenicity under conditions of chronic oral usage. Based on its reported teratogenicity and testicular toxicity (Fujii and Nishimura, 1969; Tucci and Skalko, 1978; Weinberger *et al.*, 1978; Friedman *et al.*, 1979), it was also recommended that reproductive studies be included in the evaluation of theophylline.

The oral route of administration was selected because the vast majority of human exposure to this pharmaceutical agent is by the oral route. Dosed feed was initially selected as the route of administration for the 16-day studies because theophylline is only moderately soluble in water. Additional 16-day studies were conducted using corn oil gavage as the route of administration in order to mimic human therapeutic use. Gavage studies also compared the toxicity of equivalent doses of theophylline administered once versus twice daily; the latter split-dosing regimen more closely simulates human therapeutic exposure to theophylline (normally four times per day).

Based on the results of the 16-day studies, 14-week studies were designed and conducted using two oral routes of administration, dosed feed and corn oil gavage administered once daily.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF THEOPHYLLINE

Theophylline was obtained from Henley and Company, Inc. (New York, NY), in one lot (484), which was used during the 16-day, 14-week, and 2-year feed and gavage studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix I). Reports on analyses performed in support of the theophylline studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The chemical, a white powdered solid, was identified as theophylline by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity of lot 484 was determined by elemental analyses, Karl Fischer water analysis, nonaqueous titration, thin-layer chromatography, high-performance liquid chromatography, and United States Pharmacopeia (USP) analyses. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for theophylline. Karl Fischer water analysis indicated 0.052% ± 0.007% water. Nonaqueous titration by two methods indicated purities of 99.3% ± 0.4% and 101.1% ± 0.7%. Thin-layer chromatography by two systems indicated a major spot and no impurities. High-performance liquid chromatography revealed a major peak and no impurities with areas greater than 0.1% relative to the major peak area. All results of USP analyses indicated that lot 484 met the USP specifications for theophylline. The overall purity was determined to be greater than 99%.

Accelerated stability studies of the bulk chemical were performed by the analytical chemistry laboratory using high-performance liquid chromatography. These studies indicated that theophylline was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature in a plastic bag in the original metal container or in amber glass bottles.

Stability was monitored by the study laboratory during the 16-day gavage studies and 14-week feed and gavage studies using high-performance liquid chromatography and nonaqueous titration as a weak acid, and during the 2-year studies using high-performance liquid chromatography. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Feed Studies

The dose formulations were prepared twice during the 16-day studies and weekly during the 14-week studies by mixing theophylline with feed (Table I1). Homogeneity studies of the 1,000 and 4,000 ppm dose formulations were performed by the study laboratory using high-performance liquid chromatography. The analytical chemistry laboratory conducted homogeneity studies using ultraviolet/visible spectroscopy and stability studies using high-performance liquid chromatography on the 1,000 ppm dose formulation and on a 10,000 ppm formulation. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for at least 21 days at -20° C when protected from air and light and was confirmed for at least 7 days at room temperature when exposed to air and light.

Periodic analyses of the dose formulations of theophylline were conducted at the study laboratory using high-performance liquid chromatography. During the 16-day studies, dose formulations were analyzed once (Table I2). For the 14-week studies, the initial, middle, and final dose preparations were analyzed (Table I3). All dose formulations analyzed and used during the 16-day and 14-week feed studies were within 10% of the target concentration. Results of two referee analyses performed by the analytical

chemistry laboratory agreed with the results for the 14-week studies obtained by the study laboratory (Table I4).

Gavage Studies

The dose formulations were prepared twice during the 16-day studies, weekly during the 14-week studies, and every 2 weeks during the 2-year studies by mixing theophylline with corn oil to give the required concentrations (Table I1). Homogeneity studies of the 1.36 and 87.1 mg/g dose formulations used during the 16-day studies and the 0.82 and 16.3 mg/g dose formulations used during the 2-year studies were performed by the study laboratory using ultraviolet/visible spectroscopy (250 to 290 nm). The analytical chemistry laboratory also performed homogeneity testing on a 100.1 mg/mL suspension using ultraviolet/visible spectroscopy (270 nm). A stability study conducted at the analytical chemistry laboratory on a 1 mg/mL (1.1 mg/g) theophylline in corn oil suspension was performed using high-performance liquid chromatography. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for at least 21 days at 5° C and at room temperature when stored in sealed vessels protected from light and for at least 3 hours when stored exposed to air and light.

Periodic analyses of the dose formulations of theophylline were conducted at the study laboratory using ultraviolet/visible spectroscopy (16-day and 14-week studies) or visible spectroscopy (2-year studies). During the 16-day studies, dose formulations were analyzed once (Table I5). For the 14-week studies, dose formulations from the beginning, middle, and end of the studies were analyzed (Table I6). During the 2-year studies, dose formulations were analyzed approximately every 6 to 10 weeks (Table I7). All dose formulations analyzed and used during the 16-day, 14-week, and 2-year studies were within 10% of the target concentration. In addition to dose formulation analysis prior to dosing, samples collected after dosing (animal room samples) were analyzed periodically. All animal room samples from dose formulations used during the 16-day and 14-week studies were within 10% of the target concentration. For the 2-year studies, 84% were within 10% of the target concentration. The remaining five samples ranged from 28% to 112% of the target concentration. Results of periodic referee analyses performed by the analytical chemistry laboratory during the 14-week studies agreed with the results obtained by the study laboratory (Table I8). Periodic analyses of the corn oil vehicle by the study laboratory demonstrated that peroxide concentrations were within the acceptable limit of 10 mEq/kg designated for the 16-day and 14-week studies. For the 2-year studies, the maximum acceptable limit for peroxide was 3 mEq/kg, and all samples were below this concentration with the exception of two lots. One lot that was slightly above the acceptable peroxide concentration was used for dosing until another lot of corn oil could be obtained.

16-DAY FEED STUDIES

The 16-day feed studies were conducted to determine target organ toxicity and the dose concentrations to be used in the 14-week feed studies. Male and female F344/N rats and B6C3F$_1$ mice were obtained from Frederick Cancer Research Facility (Frederick, MD). Animals were held for 12 days and were 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice were fed diets containing 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm theophylline. Feed and water were available *ad libitum*. Rats and mice were housed five per cage. Clinical findings were recorded twice daily for rats and mice. Feed consumption was measured daily. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 16-day studies, animals were anesthetized with ether and blood was collected for hematology analyses from the caudal artery or abdominal aorta of rats and by cardiac puncture of mice. Blood was transferred to tubes containing EDTA as an anticoagulant. Hematology analyses were performed on an Ortho ELT-8 hematology analyzer (Ortho Instruments, Westwood, MA). The parameters measured are listed in Table 1. A necropsy was performed on all rats and mice. The brain, heart, right kidney, liver, lung, right ovary, right testis, thymus, and uterus were weighed. Histopathologic examinations were performed on the uteri of all female rats. Table 1 lists the tissues and organs examined.

14-WEEK FEED STUDIES

The 14-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to theophylline and to determine the appropriate doses to be used in the 2-year studies.

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Rats were held for 11 days and averaged 6 weeks old on the first day of the study; mice were held for 15 days and averaged 7 weeks old on the first day of the study. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were fed diets containing 0, 1,000, 2,000, or 4,000 ppm theophylline. Feed and water were available *ad libitum*. Rats were housed five per cage and mice were housed individually. Animals were observed twice daily for signs of toxicity or moribundity. Feed consumption was measured weekly by cage. Body weights and clinical findings were recorded initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 14-week studies, animals were anesthetized with carbon dioxide and blood was collected from the retroorbital sinus of all rats and mice for hematology analyses. Methods for hematology analyses were the same as those described for the 16-day feed studies. In addition, differential leukocyte counts, reticulocyte counts, and morphologic evaluation of blood cells were determined by light microscopic examination of blood smears stained with buffered Wright-Giemsa. The parameters measured are listed in Table 1.

At the end of the 14-week studies, samples were collected from all groups of rats and mice for sperm morphology and vaginal cytology evaluations. The parameters evaluated are listed in Table 1. Methods used were those described in NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1984). For 7 consecutive days prior to the end of the studies, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm morphology, count, and motility. The right testis and right epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each right cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. Four sperm morphology slides were prepared for each animal evaluated. An aliquot of killed sperm suspension was stained in a test tube, spread on a microscope slide under a coverslip, and examined.

A necropsy was performed on all animals. The brain, heart, liver, lungs, right kidney, right ovary, right testis, thymus, and uterus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all 0 ppm and 4,000 ppm rats and mice and on selected tissues in the 1,000 ppm and 2,000 ppm rats and mice. Table 1 lists the tissues and organs routinely examined.

16-DAY GAVAGE STUDIES

Palatability problems were suspected during the 16-day feed studies; therefore, 16-day studies were conducted changing the route of administration to gavage to determine target organ toxicity and the dose concentrations to be used during the 14-week gavage studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Frederick Cancer Research Facility (Frederick, MD). On receipt, the rats and mice were approximately 4 weeks old. Males were quarantined for 13 days and females for 14 days. Animals were approximately 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice received theophylline in corn oil by gavage at doses of 0, 12.5 (twice daily), 25, 50, 50 (twice daily), 100, 200, 200 (twice daily), or 400 mg/kg. Feed and water were available *ad libitum*. Rats were housed five per cage and mice were housed individually. Feed consumption was recorded for the first week. Clinical findings were recorded twice daily. The animals were weighed on days 1, 8, and 15, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 16-day studies, all animals were anesthetized with ether and blood was collected from the inferior vena cava of rats and by cardiac puncture of mice for hematology analyses. Blood was transferred to tubes containing EDTA. Hematocrit, hemoglobin concentration, erythrocyte and leukocyte counts, mean cell hemoglobin, mean cell hemoglobin concentration, and mean cell volume were measured using the Ortho ELT-8 hematology analyzer. Differential leukocyte counts and reticulocyte counts were determined by light microsopic examination of blood smears stained with Wright-Giemsa. The hematology parameters measured are listed in Table 1.

A necropsy was performed on all rats and mice. The brain, heart, right kidney, liver, lungs, right ovary, right testis, thymus, and uterus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on vehicle control, 200, 200 (twice daily), and 400 mg/kg rats and mice. Also, the uteri of one rat and one mouse each from the 25 and 50 mg/kg groups were examined, and tissues adjacent to the mesenteric lymph nodes were examined in all dose groups. Table 1 lists the tissues and organs examined.

14-WEEK GAVAGE STUDIES

The 14-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to theophylline and to determine the appropriate doses to be used in the 2-year studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Rats were quarantined for 11 days and were approximately 6 weeks old on the first day of the study. Mice were quarantined for 15 days and were approximately 7 weeks old on the first day of the study. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats received theophylline in corn oil by gavage at doses of 0, 37.5, 75, or 150 mg/kg. Groups of 10 male and 10 female mice received theophylline in corn oil by gavage at doses of 0, 75, 150, or 300 mg/kg. Feed and water were available *ad libitum*. Rats were housed five per cage and mice were housed individually. Animals were observed twice daily for mortality and signs of toxicity. Feed consumption was recorded weekly. Body weights and clinical findings were recorded initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the studies, all animals were anesthetized with carbon dioxide and blood samples were collected from the retroorbital sinus for hematology analyses. Methods used for hematology analyses were the

same as those described for the 16-day gavage studies. The parameters measured are listed in Table 1.

At the end of the 14-week studies, samples were collected from all groups of rats and mice for sperm morphology and vaginal cytology evaluations. The parameters evaluated are listed in Table 1. Methods used were those described in NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1984). For 7 consecutive days prior to the end of the studies, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm morphology, count, and motility. The right epididymis and right testis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each right cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. Four sperm morphology slides were prepared for each animal evaluated. An aliquot of killed sperm suspension was stained in a test tube, spread on a microscope slide under a coverslip, and examined.

A necropsy was performed on all animals. The brain, heart, right kidney, liver, lungs, right ovary, right testis, thymus, and uterus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 to 6 µm, and stained withhematoxylin and eosin. Complete histopathologic examinations were performed on vehicle controls, 150 mg/kg rats, 150 mg/kg female mice, and 300 mg/kg mice. Additionally, mesenteric tissue adjacent to the lymph nodes and pancreas was examined for all rats in all dose groups. The kidneys, lungs, spleen, thymus, and urinary bladder from 150 mg/kg male mice, the kidneys from 75 mg/kg male mice, and the liver and heart from 75 mg/kg female mice were examined. Table 1 lists the tissues and organs routinely examined.

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and 50 female mice received theophylline in corn oil by gavage at doses of 0, 7.5, 25, or 75 mg/kg. Groups of 50 male mice received theophylline in corn oil by gavage at doses of 0, 15, 50, or 150 mg/kg.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA) for use in the 2-year studies. Rats and mice were quarantined for 14 days and 11 days, respectively, before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats were housed five per cage and mice were housed individually. Feed and water were available *ad libitum*. Cages were rotated once every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Rats were weighed initially, weekly for the first 13 weeks, approximately every 4 weeks thereafter, and at the end of the study. Mice were weighed initially, weekly for the first 15 weeks, approximately every 4 weeks thereafter, and at the end of the study. Clinical

findings were recorded initially, approximately every 4 weeks, and at the end of the studies.

A complete necropsy and microscopic examination were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (i.e., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist reviewed selected slides from rats of the brain, kidney, liver, lung, mesentery, nose, oral mucosa, pancreas, pituitary gland, skin, spleen, and uterus. The quality assessment pathologist reviewed selected slides from mice of the adrenal cortex, forestomach, epididymis, gallbladder, kidney, liver, pituitary gland, small intestine, spleen, thymus, and thyroid gland.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to theophylline administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologist, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al*. (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al*. (1986).

TABLE 1
Experimental Design and Materials and Methods in the Feed and Gavage Studies of Theophylline

16-Day Feed Studies	14-Week Feed Studies
Study Laboratory	
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)
Strain and Species	
Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$
Animal Source	
Frederick Cancer Research Facility (Frederick, MD)	Taconic Farms (Germantown, NY)
Time Held Before Studies	
12 days	Rats: 11 days Mice: 15 days
Average Age When Studies Began	
41 days	Rats: 42 days Mice: 46 days
Date of First Dose	
Rats: 28 November 1983 Mice: 21 November 1983	Rats: 31 March 1986 Mice: 4 April 1986
Duration of Dosing	
Rats: 15 days (7 days/week) (males) 16 days (7 days/week) (females) Mice: 14 days (7 days/week) (males) 15 days (7 days/week) (females)	Rats: 92 to 94 days (7 days/week) Mice: 95 to 97 days (7 days/week)
Date of Last Dose	
Rats: 12 December 1983 (males) 13 December 1983 (females) Mice: 4 December 1983 (males) 5 December 1983 (females)	Rats: 30 June to 2 July 1986 Mice: 7 to 9 July 1986
Necropsy Dates	
Rats: 13 December 1983 (males) 14 December 1983 (females) Mice: 5 December 1983 (males) 6 December 1983 (females)	Rats: 30 June to 2 July 1986 Mice: 7 to 9 July 1986
Average Age at Necropsy	
Rats: 56 days (males) 57 days (females) Mice: 55 days (males) 56 days (females)	Rats: 133 to 135 days Mice: 140 to 142 days
Size of Study Groups	
5 males and 5 females	10 males and 10 females

TABLE 1
Experimental Design and Materials and Methods in the Feed and Gavage Studies of Theophylline (continued)

16-Day Gavage Studies	14-Week Gavage Studies	2-Year Gavage Studies
Study Laboratory		
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)
Strain and Species		
Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$
Animal Source		
Frederick Cancer Research Facility (Frederick, MD)	Taconic Farms (Germantown, NY)	Simonsen Laboratories, Inc. (Gilroy, CA)
Time Held Before Studies		
13 days (males) 14 days (females)	Rats: 11 days Mice: 15 days	Rats: 14 days Mice: 11 days
Average Age When Studies Began		
42 days (males) 43 days (females)	Rats: 42 days Mice: 46 days	Rats: 43 days Mice: 40 days
Date of First Dose		
Rats: 11 June (males) or 12 June (females) 1985 Mice: 4 June (males) or 5 June (females) 1985	Rats: 14 April 1986 Mice: 18 April 1986	Rats: 25 October 1990 Mice: 15 October 1990
Duration of Dosing		
16 days (5 days/week)	Rats: 93 to 95 days (5 days/week) Mice: 96 to 98 days (5 days/week)	Rats: 729 days (5 days/week) Mice: 726 days (5 days/week)
Date of Last Dose		
Rats: 26 June (males) or 27 June (females) 1985 Mice: 19 June (males) or 20 June (females) 1985	Rats: 15 to 17 July 1986 Mice: 22 to 24 July 1986	Rats: 22 October 1992 Mice: 9 October 1992
Necropsy Dates		
Rats: 27 June (males) or 28 June (females) 1985 Mice: 20 June (males) or 21 June (females) 1985	Rats: 16 to 18 July 1986 Mice: 23 to 25 July 1986	Rats: 22 to 27 October 1992 (males) 22 to 28 October 1992 (females) Mice: 8 and 12 to 14 October 1992 (males) 8, 9, and 14 to 16 October 1992 (females)
Average Age at Necropsy		
Rats: 58 days (males) 59 days (females) Mice: 58 days (males) 59 days (females)	Rats: 135 to 137 days Mice: 142 to 144 days	Rats: 111 weeks Mice: 110 to 111 weeks
Size of Study Groups		
5 males and 5 females	10 males and 10 females	50 males and 50 females

TABLE 1
Experimental Design and Materials and Methods in the Feed and Gavage Studies of Theophylline (continued)

16-Day Feed Studies	14-Week Feed Studies
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 16-day feed studies
Animals per Cage	
Rats: 5 Mice: 5	Rats: 5 Mice: 1
Method of Animal Identification	
Toe clip	Toe clip
Diet	
NIH-07 open formula mash (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 16-day feed studies
Water Distribution	
Tap water (Birmingham, AL) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 16-day feed studies
Cages	
Solid-bottom polycarbonate (Lab Products, Inc., Garfield, NJ); changed twice a week, not rotated	Solid-bottom polycarbonate (Lab Products, Inc., Maywood, NJ); changed at least once a week, rotated once every 2 weeks
Bedding	
BetaChips® heat-treated hardwood chips (Northeastern Products Corp., Warrensburg, NY); changed twice weekly	Same as 16-day feed studies
Cage Filters	
Reemay® spun-bonded polyester cage filters (Snow Filtration, Cincinnati, OH); changed once every 2 weeks	Reemay® spun-bonded polyester cage filters (Andico, Birmingham, AL); changed once every 2 weeks
Racks	
Stainless steel (Lab Products, Inc., Garfield, NJ); changed once every 2 weeks, not rotated	Stainless steel (Lab Products, Inc., Maywood, NJ); changed and rotated once every 2 weeks
Animal Room Environment	
Temperature: 21° to 23° C Relative humidity: 38% to 54% (rats) or 40% to 50% (mice) Fluorescent light: 12 hours/day Room air: 10 changes/hour, minimum	Temperature: 20° to 25° C Relative humidity: 34% to 64% Fluorescent light: 12 hours/day Room air: 10 changes/hour, minimum

TABLE 1
Experimental Design and Materials and Methods in the Feed and Gavage Studies of Theophylline (continued)

16-Day Gavage Studies	14-Week Gavage Studies	2-Year Gavage Studies
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 16-day gavage studies	Same as 16-day gavage studies
Animals per Cage Rats: 5 Mice: 1	Rats: 5 Mice: 1	Rats: 5 Mice: 1
Method of Animal Identification Toe clip	Toe clip	Tail tattoo
Diet NIH-07 Open Formula Pellets (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 16-day gavage studies	Same as 16-day gavage studies
Water Distribution Tap water (Birmingham, AL) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 16-day gavage studies	Same as 16-day gavage studies
Cages Solid-bottom polycarbonate (Lab Products, Inc., Garfield, NJ); changed twice weekly, not rotated	Solid-bottom polycarbonate (Lab Products, Inc., Maywood, NJ); changed at least once a week; rotated once every 2 weeks	Solid-bottom polycarbonate (Lab Products, Inc., Maywood, NJ); rat cages were changed twice weekly and mouse cages were changed once weekly; rotated once every 2 weeks
Bedding BetaChips® heat-treated hardwood chips (Northeastern Products Corp., Warrensburg, NY); changed twice weekly	Same as 16-day gavage studies	Sani-Chips® heat-treated hardwood chips (Murphy Forest Products Corp., Montville, NJ); rat bedding was changed twice weekly and mouse bedding was changed once weekly
Cage Filters Reemay® spun-bonded polyester (Snow Filtration, Cincinnati, OH); changed once every 2 weeks	Reemay® spun-bonded polyester (Andico, Birmingham, AL); changed once every 2 weeks	Same as 14-week gavage studies
Racks Stainless steel (Lab Products, Inc., Garfield, NJ); changed once every 2 weeks, not rotated	Stainless steel (Lab Products, Inc., Maywood, NJ); changed and rotated once every 2 weeks	Same as 14-week gavage studies
Animal Room Environment Temperature: 21° to 25° C Relative humidity: 43% to 59% (rats) or 35% to 70% (mice) Fluorescent light: 12 hours/day Room air: 10 changes/hour, minimum	Temperature: 20° to 25° C Relative humidity: 34% to 66% Fluorescent light: 12 hours/day Room air: 10 changes/hour, minimum	Temperature: 17° to 26° C (rats) or 13° to 25° C (mice) Relative humidity: 26% to 86% (rats), 15% to 90% (mice) Fluorescent light: 12 hours/day Room air: 10 changes/hour, minimum

TABLE 1
Experimental Design and Materials and Methods in the Feed and Gavage Studies of Theophylline (continued)

16-Day Feed Studies	14-Week Feed Studies
Doses	
0, 500, 1,000, 2,000, 4,000, or 8,000 ppm in feed, available *ad libitum*	0, 1,000, 2,000, or 4,000 ppm in feed, available *ad libitum*
Type and Frequency of Observation	
Observed and clinical findings recorded twice daily; body weights were recorded on days 1, 8, and 15 (rats) or 14 (mice), and at the end of the studies. Feed consumption was measured daily and calculated weekly by cage.	Observed twice daily; body weights and clinical findings were recorded initially, weekly, and at the end of the studies; feed consumption was measured weekly by cage.
Method of Sacrifice	
Rats: CO_2 asphyxiation Mice: Ether anesthesia followed by opening of the thoracic cavity	CO_2 asphyxiation
Necropsy	
Necropsy was performed on all animals. Organs weighed were brain, heart, right kidney, liver, lungs, right ovary, right testis, thymus, and uterus.	Necropsy was performed on all animals. Organs weighed were brain, heart, right kidney, liver, lungs, right ovary, right testis, thymus, and uterus.
Clinical Pathology	
Blood was obtained for hematology from the caudal artery or abdominal aorta of rats or by cardiac puncture of mice following ether anesthesia. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte count; and leukocyte count	Blood for hematology was collected from the retroorbital sinus of all animals. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte count; reticulocyte count; nucleated erythrocyte count (rats); mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count; leukocyte count and differential; and atypical leukocyte count (rats)

TABLE 1
Experimental Design and Materials and Methods in the Feed and Gavage Studies of Theophylline (continued)

16-Day Gavage Studies	14-Week Gavage Studies	2-Year Gavage Studies
Doses		
0, 12.5 (twice daily), 25, 50, 50 (twice daily), 100, 200, 200 (twice daily), or 400 mg/kg in corn oil by gavage	Rats: 0, 37.5, 75, or 150 mg/kg in corn oil by gavage Mice: 0, 75, 150, or 300 mg/kg in corn oil by gavage	Rats: 0, 7.5, 25, or 75 mg/kg in corn oil by gavage Mice: 0, 15, 50, or 150 mg/kg (males) and 0, 7.5, 25, or 75 mg/kg (females) in corn oil by gavage
Dose Volumes		
Rats: 5 mL/kg body weight Mice: 10 mL/kg body weight	Rats: 5 mL/kg body weight Mice: 10 mL/kg body weight	Rats: 5 mL/kg body weight Mice: 10 mL/kg body weight
Type and Frequency of Observation		
Observed and clinical findings recorded twice daily; body weights were recorded on days 1, 8, and 15, and at the end of the studies. Feed consumption was recorded for the first week.	Observed twice daily; body weights and clinical findings were recorded initially, weekly, and at the end of the studies; feed consumption was recorded weekly.	Observed twice daily; weighed initially, approximately weekly for the first 13 weeks (rats) or 15 weeks (mice), approximately every 4 weeks thereafter, and at the end of the studies; clinical findings were recorded initially, approximately every 4 weeks, and at the end of the studies.
Method of Sacrifice		
Ether anesthesia followed by opening of the thoracic cavity	CO_2 asphyxiation	CO_2 asphyxiation
Necropsy		
Necropsy was performed on all animals. Organs weighed were brain, heart, right kidney, liver, lungs, right ovary, right testis, thymus, and uterus.	Necropsy was performed on all animals. Organs weighed were brain, heart, right kidney, liver, lungs, right ovary, right testis, thymus, and uterus.	Necropsy was performed on all animals.
Clinical Pathology		
Blood was collected from all surviving animals from the inferior vena cava of rats or by cardiac puncture in mice following ether anesthesia. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte and reticulocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count; and leukocyte count and differential	Blood was collected from all surviving animals from the retroorbital sinus for hematology. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte and reticulocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count; and leukocyte count and differential	None

TABLE 1
Experimental Design and Materials and Methods in the Feed and Gavage Studies of Theophylline (continued)

16-Day Feed Studies	14-Week Feed Studies
Histopathology	
The uterus of each female rat was examined.	Complete histopathology was performed on all 0 ppm and 4,000 ppm animals. In addition to gross lesions and tissue masses, the tissues examined included: adrenal glands, bone and marrow, brain, clitoral gland (rats), esophagus, gallbladder (mice), harderian gland (female rats), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovaries, pancreas, parathyroid glands, pituitary gland, preputial gland (rats), prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testes (and epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. Tissue adjacent to the mesenteric lymph nodes was examined from all groups of rats. Additionally, in 1,000 and 2,000 ppm rats, heart, kidney, and preputial gland of males, and the harderian gland and pancreas of females were examined. In 1,000 and 2,000 ppm mice, the liver of males and females and mammary gland of females were examined.
Sperm Morphology and Vaginal Cytology Evaluation	
None	At the end of the studies, sperm samples were collected from all males for sperm morphology evaluations. The following parameters were evaluated: sperm motility, percent abnormal sperm, and sperm concentration. The right cauda, right epididymis, and right testis were weighed. Vaginal samples were collected for up to 7 consecutive days prior to the end of the studies from all females for vaginal cytology evaluations. The following parameters were evaluated: relative frequency of estrous stages and estrous cycle length.

TABLE 1
Experimental Design and Materials and Methods in the Feed and Gavage Studies of Theophylline (continued)

16-Day Gavage Studies	14-Week Gavage Studies	2-Year Gavage Studies
Histopathology		
Complete histopathology was performed on vehicle control, 200 (once daily), 200 (twice daily), and 400 mg/kg (once daily) rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal glands, bone and marrow, brain, clitoral gland (rats), esophagus, gall bladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovaries, pancreas, parathyroid glands, pituitary gland, preputial gland (rats), prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testes (and epididymis and seminal vesicle), thymus, thyroid glands, trachea, urinary bladder, and uterus.	Complete histopathology was performed on vehicle controls, 150 mg/kg rats, 150 mg/kg female mice, and 300 mg/kg mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal glands, bone and marrow, brain, clitoral gland (rats), esophagus, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovaries, pancreas, parathyroid glands, pituitary gland, preputial gland (rats), prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testes (and epididymis and seminal vesicle), thymus, thyroid glands, trachea, urinary bladder, and uterus. Additionally, in rats, mesenteric tissue adjacent to lymph nodes and the pancreas was examined from all dose groups; in mice, the kidneys of 75 mg/kg males, the heart and liver of 75 mg/kg females, and the kidneys, lungs, spleen, thymus, and urinary bladder of 150 mg/kg males were examined.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal glands, bone and marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovaries, pancreas, parathyroid glands, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testes (and epididymis and seminal vesicle), thymus, thyroid glands, trachea, urinary bladder, and uterus. Additionally, mesenteric tissue was examined from all male and female rats.
Sperm Morphology and Vaginal Cytology Evaluation		
None	At the end of the studies, sperm samples were collected from all males for sperm morphology evaluations. The following parameters were evaluated: sperm motility, percent abnormal sperm, and sperm concentration. The right cauda, right epididymis, and right testis were weighed. Vaginal samples were collected for up to 7 consecutive days prior to the end of the studies from all females for vaginal cytology evaluations. The following parameters were evaluated: relative frequency of estrous stages and estrous cycle length.	None

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A5, B1, B5, C1, C5, D1, and D5 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical signifi-cance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm, i.e., the Kaplan-Meier estimate of the neoplasm incidence that would have been observed at the end of the study in the absence of mortality from all other competing risks (Kaplan and Meier, 1958).

Analysis of Neoplasm Incidences

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

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

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

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett

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

Historical Control Data

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

QUALITY ASSURANCE METHODS

The 14-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of theophylline was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells and mouse bone marrow cells, and increases in the frequency of micronucleated normochromatic erythrocytes in mouse peripheral blood. The protocols for these studies and the results are given in Appendix E.

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

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests correlate less well with rodent carcinogenicity (Tennant *et al*., 1987; Zeiger *et al*., 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests.

That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone.

The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests appears to be less than the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). Positive responses in long term peripheral blood micronucleus tests have not been formally evaluated for their predictivity of rodent carcinogenicity. But, because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

RESULTS

RATS

16-DAY FEED STUDY

All rats survived until the end of the study (Table 2). The mean body weight gain of females exposed to 1,000 ppm was significantly greater than that of the control group, while the final mean body weights and body weight gains of 8,000 ppm males and females were significantly less than those of the controls. Feed consumption by exposed groups was similar to that by the controls; however, feed was observed piled under the feeders of males and females in the 8,000 ppm groups. Dietary levels of 500, 1,000, 2,000, 4,000, or 8,000 ppm resulted in approximate daily doses of 50, 100, 250, 450, or 1,000 mg theophylline/kg body weight to males and 75, 150, 250, 450, or 1,100 mg/kg to females. No clinical findings were attributed to theophylline exposure.

Hematocrit values, hemoglobin concentrations, and erythrocyte counts were significantly increased in males exposed to 2,000, 4,000, or 8,000 ppm (Table G1). Hematocrit values and hemoglobin concentrations were significantly increased in females exposed to 500, 2,000, 4,000, or 8,000 ppm. The hematology differences in exposed rats were considered to be manifestations of hemoconcentration resulting from the known diuretic effect of theophylline.

TABLE 2
Survival, Body Weights, and Feed Consumption of Rats in the 16-Day Feed Study of Theophylline

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2[d]
Male							
0	5/5	128 ± 2	191 ± 5	63 ± 3		123.9	106.2
500	5/5	132 ± 2	209 ± 5	77 ± 4	110	117.3	97.8
1,000	5/5	125 ± 3	194 ± 4	70 ± 4	102	114.4	101.6
2,000	5/5	124 ± 1	191 ± 5	67 ± 5	100	132.3	113.5
4,000	5/5	127 ± 3	186 ± 4	59 ± 2	97	108.3	117.2
8,000	5/5	126 ± 3	141 ± 4**	15 ± 2**	74	122.5	129.9
Female							
0	5/5	103 ± 1	137 ± 2	34 ± 2		126.3	114.9
500	5/5	103 ± 2	143 ± 4	40 ± 2	104	156.4	128.9
1,000	5/5	102 ± 1	145 ± 2	43 ± 2*	106	176.4	122.8
2,000	5/5	102 ± 1	140 ± 4	38 ± 3	102	131.8	109.9
4,000	5/5	102 ± 1	141 ± 2	39 ± 2	103	114.4	103.0
8,000	5/5	104 ± 1	119 ± 2**	16 ± 1**	87	159.1	115.4

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per kilogram animal weight per day. True feed consumption for the high-dose males and females is unknown because feed was observed piled under the feeders. It was speculated that the rats found the feed unpalatable and kicked it out of the feeder.
[d] Eight days in week 2 for females

The absolute and relative heart and liver weights of 8,000 ppm males and the absolute heart weight of 8,000 ppm females were significantly less than those of the controls (Table F1). The relative testis weights of 2,000, 4,000, and 8,000 ppm males were significantly greater than that of the control group, as were the absolute testis weights of 4,000 ppm males.

At necropsy, small uteri were observed in exposed groups, and microscopic examination revealed a dose-dependent increase in incidences of uterine hypoplasia (0 ppm, 0/5; 500 ppm, 1/5; 1,000 ppm, 1/5; 2,000 ppm, 2/5; 4,000 ppm, 3/5; 8,000 ppm, 4/5). Two 8,000 ppm males had small seminal vesicles; one of these rats also had small testes.

16-DAY GAVAGE STUDY

All rats receiving 400 mg/kg once daily and all rats receiving 200 mg/kg twice daily except one female died during the study (Tables 3 and 4). In the groups dosed once daily, the final mean body weights and body weight gains of 100 and 200 mg/kg males and mean body weight gains of 50, 100, and 200 mg/kg females were significantly less than those of the controls. In comparing groups that received the same daily dosage administered in once-daily or twice-daily doses, the final mean body weights and body weight gains were similar. However, males receiving 25 mg/kg once daily had a significantly greater final mean body weight and body weight gain than males receiving 12.5 mg/kg twice daily. Animals receiving the lethal doses (200 mg/kg twice daily or 400 mg/kg once daily) experienced rapid respiration, labored respiration, rigid bodies with tremors, hunched posture, and squinting.

TABLE 3
Survival and Body Weights of Rats in the 16-Day Gavage Study of Theophylline: Comparison of Groups Receiving Once-Daily Administration

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	137 ± 2	212 ± 3	75 ± 3	
25	5/5	139 ± 3	213 ± 5	74 ± 3	100
50	5/5	136 ± 4	201 ± 6	65 ± 4	95
100	5/5	131 ± 2	189 ± 4**	58 ± 4**	89
200	5/5	141 ± 3	177 ± 4**	36 ± 4**	84
400	0/5[c]	143 ± 2	—	—	—
Female					
0	5/5	105 ± 2	141 ± 4	37 ± 2	
25	5/5	108 ± 2	140 ± 4	32 ± 2	99
50	5/5	107 ± 1	136 ± 1	29 ± 1*	97
100	5/5	103 ± 2	135 ± 2	32 ± 2*	96
200	5/5	107 ± 1	132 ± 2	24 ± 2**	93
400	0/5[d]	106 ± 2	—	—	—

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. No final mean body weights were calculated for groups with 100% mortality.
[c] Day of death: 1, 3, 3, 4, 4
[d] Day of death: 3, 3, 4, 4, 14

TABLE 4
Survival and Body Weights of Rats in the 16-Day Gavage Study of Theophylline: Comparisons of Once-Daily to Twice-Daily Administration

Dose (mg/kg)		Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Twice-Daily Group (%)
			Initial	Final	Change	
Male						
Low-Dose Comparison						
12.5	twice daily	5/5	133 ± 3	194 ± 5	62 ± 3	
25	once daily	5/5	139 ± 3	213 ± 5*	74 ± 3*	109
Mid-Dose Comparison						
50	twice daily	5/5	132 ± 4	192 ± 5	59 ± 2	
100	once daily	5/5	131 ± 2	189 ± 4	58 ± 4	98
High-Dose Comparison						
200	twice daily	0/5[c]	—	—	—	
400	once daily	0/5[d]	—	—	—	
Female						
Low-Dose Comparison						
12.5	twice daily	5/5	103 ± 1	132 ± 2	29 ± 3	
25	once daily	5/5	108 ± 2*	140 ± 4	32 ± 2	106
Mid-Dose Comparison						
50	twice daily	5/5	107 ± 1	138 ± 2	31 ± 2	
100	once daily	5/5	103 ± 2	135 ± 2	32 ± 2	98
High-Dose Comparison						
200	twice daily	1/5[e]	—	—	—	
400	once daily	0/5[f]	—	—	—	

* Significantly different ($P \leq 0.05$) from the twice-daily administration group by a *t*-test
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. No final mean body weights were calculated for groups with 100% mortality. No standard errors were calculated for groups with high mortality.
[c] Day of death: 3, 4, 5, 5, 11
[d] Day of death: 1, 3, 3, 4, 4
[e] Day of death: 2, 2, 9, 9
[f] Day of death: 3, 3, 4, 4, 14

In groups dosed once daily, the hemoglobin concentration of males receiving 200 mg/kg and the hematocrit value, hemoglobin concentration, and erythrocyte count of females receiving 200 mg/kg were significantly greater than those of the respective control groups (Table G2). There were no biologically significant differences in hematology parameters between groups with equivalent daily dosage (Table G3).

In groups dosed once daily, the absolute thymus weight of males receiving 200 mg/kg and absolute and relative thymus weights of females receiving 100 or 200 mg/kg were significantly less than those of the respective controls (Table F2). The absolute and relative uterus weights of females receiving 100 or 200 mg/kg once daily were significantly less than those of the control group, and the absolute and relative uterus weights of females receiving

100 mg/kg once daily were significantly less than those of females receiving 50 mg/kg twice daily (Table F3).

Acute to subacute periarteritis of minimal to moderate severity was observed in the medium-sized mesenteric arteries adjacent to the mesenteric lymph nodes of two male and two female rats given 400 mg/kg once daily and adjacent to the pancreas of one of these two males (Table 5). Arterial changes were segmental or circumferential and consisted of medial hemorrhage and fibrinoid necrosis. The adventitia contained a mixed inflammatory cell infiltrate consisting of neutrophils, macrophages, mononuclear cells, and proliferating fibroblasts (Plates 1, 2, and 3). Minimal to mild necrosis and/or subacute inflammation were observed in the hearts of three males given 200 mg/kg twice daily, one female given 200 mg/kg once daily, and one female given 400 mg/kg once daily. These lesions are consistent with the known cardiotoxic effects of theophylline. Acute inflammation, edema, erosions, ulcers, and/or mucosal hyperplasia of the forestomach and/or glandular stomach were observed in low numbers of males given 100 mg/kg once daily, 200 mg/kg twice daily, or 400 mg/kg once daily and in females in the control group and groups given 200 mg/kg twice daily or 400 mg/kg once daily. These lesions may be related to the known gastrointestinal effects of theophylline or secondary to gavage-induced trauma. Uterine atrophy was noted in three females receiving 200 mg/kg twice daily. Males and females receiving 200 mg/kg twice daily or 400 mg/kg once daily and that died before the end of the study had lung congestion considered to be a nonspecific change accompanying agonal death and not a direct compound-related effect. Lymphoid depletion observed in the spleen and thymus is also a common finding in moribund animals, as is bone marrow depletion noted in two males and one female receiving 200 mg/kg twice daily.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Rats in the 16-Day Gavage Study of Theophylline

	Vehicle Control Once Daily	100 mg/kg Once Daily	200 mg/kg Once Daily	200 mg/kg Twice Daily	400 mg/kg Once Daily
Male					
Bone Marrow[a]	5	5	5	5	5
Depletion[b]	0	0	0	2 (2.5)[c]	0
Heart, Myocardium	5	5	5	5	5
Subacute Inflammation, Atrium	0	0	0	1 (4.0)	0
Subacute Inflammation, Ventricle	0	0	0	3 (1.7)	0
Necrosis, Ventricle	0	0	0	2 (1.5)	0
Lung	5	5	5	5	5
Congestion	0	0	0	5** (2.6)	3 (2.0)
Mesentery	—[d]	—	5	5	5
Artery, Periarteritis[e]			0	0	2 (2.0)
Spleen	5	5	5	5	5
Lymphoid Follicle, Depletion	0	0	0	5** (2.8)	3 (2.7)
Hematopoietic Cell, Proliferation	0	0	0	0	2 (2.5)
Stomach, Forestomach	5	5	5	5	5
Edema	0	0	0	1 (3.0)	1 (2.0)
Erosion	0	0	0	1 (4.0)	0
Hyperplasia	0	0	0	1 (3.0)	0
Inflammation	0	0	0	1 (3.0)	1 (2.0)
Stomach, Glandular	5	5	5	5	5
Edema	0	1 (3.0)	0	0	0
Erosion	0	0	0	0	1 (2.0)
Thymus	5	5	5	5	4
Lymphoid Follicle, Depletion	0	0	0	5** (2.8)	3* (1.7)

(continued)

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Rats in the 16-Day Gavage Study of Theophylline (continued)

	Vehicle Control Once Daily	100 mg/kg Once Daily	200 mg/kg Once Daily	200 mg/kg Twice Daily	400 mg/kg Once Daily
Female					
Bone Marrow	5	5	5	5	5
Depletion	0	0	0	1 (2.0)	0
Heart, Myocardium	5	5	5	5	5
Subacute Inflammation, Ventricle	0	0	1 (1.0)	0	0
Necrosis, Ventricle	0	0	1 (1.0)	0	0
Lung	5	5	5	5	5
Congestion (2.6)	0	0	0	4* (2.8)	5**
Mesentery	—	—	5	5	5
Artery, Periarteritis[e]			0	0	2 (2.0)
Spleen	5	5	5	4	5
Lymphoid Follicle, Depletion	0	0	0	2 (2.0)	4* (2.5)
Stomach, Forestomach	5	5	5	4	5
Edema	1 (3.0)	0	0	0	1 (1.0)
Inflammation	0	0	0	0	1 (2.0)
Ulcer	0	0	0	0	1 (2.0)
Stomach, Glandular	5	5	5	5	5
Erosion	0	0	0	1 (2.0)	1 (3.0)
Ulcer	0	0	0	0	1 (3.0)
Thymus	5	5	5	5	5
Lymphoid Follicle, Depletion	0	0	0	2 (1.5)	3 (2.3)
Uterus	5	5	5	4	5
Atrophy	0	0	0	3* (2.3)	0

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with organ/tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[d] Tissue not examined at this dose level
[e] Results of microscopic reevaluation of slides from males and females in the 200 mg/kg once-daily, 200 mg/kg twice-daily, and 400 mg/kg once-daily groups.

14-WEEK FEED STUDY

All rats survived until the end of the study (Table 6). The final mean body weight of 1,000 ppm females was significantly greater than that of the controls. Feed consumption by exposed groups was similar to that by the controls. Dietary levels of 1,000, 2,000, or 4,000 ppm resulted in approximate daily doses of 75, 125, or 250 mg/kg to males and 75, 125, or 275 mg/kg to females. There were no clinical findings attributed to theophylline exposure.

TABLE 6
Survival, Body Weights, and Feed Consumption of Rats in the 14-Week Feed Study of Theophylline

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 2	Week 13
Male							
0	10/10	117 ± 2	345 ± 8	228 ± 7		95.5	44.1
1,000	10/10	116 ± 3	354 ± 5	238 ± 5	103	99.4	46.6
2,000	10/10	114 ± 2	352 ± 4	238 ± 3	102	98.5	46.8
4,000	10/10	117 ± 2	341 ± 4	224 ± 4	99	95.4	44.6
Female							
0	10/10	113 ± 6	201 ± 3	88 ± 6		87.9	51.3
1,000	10/10	117 ± 7	213 ± 3*	96 ± 5	106	85.0	51.1
2,000	10/10	112 ± 6	206 ± 4	94 ± 7	103	93.1	53.9
4,000	10/10	115 ± 6	198 ± 4	83 ± 8	99	90.9	44.9

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per kilogram animal weight per day.

Mean cell volume and mean cell hemoglobin of males exposed to 2,000 or 4,000 ppm were significantly greater than those of the control group (Table G4). The platelet count of males exposed to 4,000 ppm was significantly greater than that of the controls. Segmented neutrophil counts of females exposed to 1,000, 2,000, or 4,000 ppm were significantly greater than that of the control group.

There were no significant differences between control and exposed rats in sperm morphology or vaginal cytology parameters.

The absolute and relative kidney weights of 4,000 ppm males were significantly greater than those of the controls (Table F4). The absolute and relative lung weights of females exposed to 4,000 ppm were significantly greater than those of the controls.

Exposure-related gross lesions were not evident at necropsy. Microscopically, there was a dose-related increase in the incidence of periarteritis in the small- to medium-sized mesenteric arteries adjacent to the pancreas and/or mesenteric lymph nodes (Table 7).

Periarteritis was characterized by infiltration of mononuclear and polymorphonuclear leukocytes into the media and adventitia (Plate 4), and the more severe lesions included degeneration of medial smooth muscle (Plate 5) and periarterial fibrosis. There was an exposure-related increase in the severity of nephropathy in males. Nephropathy was characterized by randomly distributed foci of tubular regeneration, dilated tubules containing eosinophilic protein casts, and focal interstitial mononuclear cell infiltrates.

TABLE 7
Incidences of Selected Nonneoplastic Lesions in Rats in the 14-Week Feed Study of Theophylline

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Male				
Kidney[a]	10	10	10	10
Nephropathy[b]	10 (1.1)[c]	10 (1.4)	10 (1.7)	10 (2.6)
Mesentery[d]	10	10	10	10
Artery, Periarteritis	0	0	2	3
Female				
Mesentery[d]	10	10	10	10
Artery, Periarteritis	0	1	1	5*

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
[a] Number of animals with organ/tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[d] Includes results from subsequent microscopic review of tissue adjacent to mesenteric lymph nodes

14-WEEK GAVAGE STUDY

One male and one female in the 150 mg/kg group died before the end of the study (Table 8). The mean body weight gain of 150 mg/kg females was significantly greater than that of the controls. There were no clinical findings attributed to theophylline treatment.

Mean cell volume of males receiving 150 mg/kg and mean cell hemoglobin of males receiving 37.5, 75, or 150 mg/kg were significantly greater than those of the controls (Table G5). There were no significant differences in sperm morphology or vaginal cytology parameters between control and dosed rats.

The absolute and relative thymus weights of males and females receiving 150 mg/kg were significantly less than those of the controls (Table F5). The absolute liver weight of females receiving 75 mg/kg and the absolute and relative liver weights of females receiving 150 mg/kg were significantly greater than those of the controls.

TABLE 8
Survival and Body Weights of Rats in the 14-Week Gavage Study of Theophylline

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	137 ± 3	328 ± 5	190 ± 3	
37.5	10/10	136 ± 4	325 ± 5	188 ± 2	99
75	10/10	133 ± 3	323 ± 4	191 ± 6	99
150	9/10[c]	138 ± 4	313 ± 4	178 ± 5	96
Female					
0	10/10	112 ± 1	201 ± 3	89 ± 3	
37.5	10/10	110 ± 2	197 ± 2	87 ± 2	98
75	10/10	112 ± 2	205 ± 3	93 ± 2	102
150	9/10[d]	111 ± 2	209 ± 3	98 ± 2*	104

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Week of death: 1
[d] Week of death: 13

Significant treatment-related gross lesions were not evident at necropsy. A subsequent microscopic review of the mesentery and associated tissues from all animals revealed a slight dose-dependent increase in the incidence of periarteritis of the small- to medium-sized arteries adjacent to the mesenteric lymph nodes of male and female rats (males: vehicle control, 1/10; 37.5 mg/kg, 1/10; 75 mg/kg, 2/10; 150 mg/kg, 5/10; females: 0/10, 2/10, 2/10, 3/10). The periarteritis was focal or circumferential and was characterized by infiltration of mononuclear and polymorphonuclear leukocytes into the media and adventitia. In some arteries, the adventitia was expanded by proliferation of connective tissue, which contained prominent endothelial-lined spaces (Plate 6). The periarteritis observed in one control male was more consistent with that commonly observed in aged rats and consisted of minimal, focal lymphocyte accumulation adjacent to the artery.

Dose Selection Rationale: Based on the deaths of rats receiving 150 mg/kg and an absence of significant findings of toxicity or life-threatening lesions in rats receiving 75 mg/kg, the doses selected for the 2-year study were 0, 7.5, 25, and 75 mg/kg for male and female rats. The gavage route of administration was selected to mimic human therapeutic use of theophylline.

2-YEAR GAVAGE STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 9 and in the Kaplan-Meier survival curves (Figure 2). There were no significant differences in survival between control and dosed groups.

Body Weights and Clinical Findings

There was a dose-related decrease in mean body weights of male and female rats. Noticeable reductions in mean body weight gain were seen as early as week 4 in 75 mg/kg males and week 29 in 25 mg/kg females (Tables 10 and 11 and Figure 3). Generally, body weight gains declined throughout the study in all dosed groups. The final mean body weights of all dosed groups of rats were significantly less than those of the control groups. There were no clinical findings attributed to theophylline treatment.

TABLE 9
Survival of Rats in the 2-Year Gavage Study of Theophylline

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	1	1	1	0
Moribund	21	11	18	13
Natural deaths	5	5	2	13
Animals surviving to study termination	23	33	29	24
Percent probability of survival at end of study[b]	47	67	59	48
Mean survival (days)[c]	674	681	659	611
Survival analysis[d]	P=0.118	P=0.084N	P=0.526N	P=0.586
Female				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	1	0	3
Moribund	17	12	14	7
Natural deaths	1	7	3	7
Animals surviving to study termination	32[e]	30	33	33
Percent probability of survival at end of study	64	61	66	70
Mean survival (days)	681	662	690	671
Survival analysis	P=0.389N	P=0.916	P=0.955N	P=0.584N

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

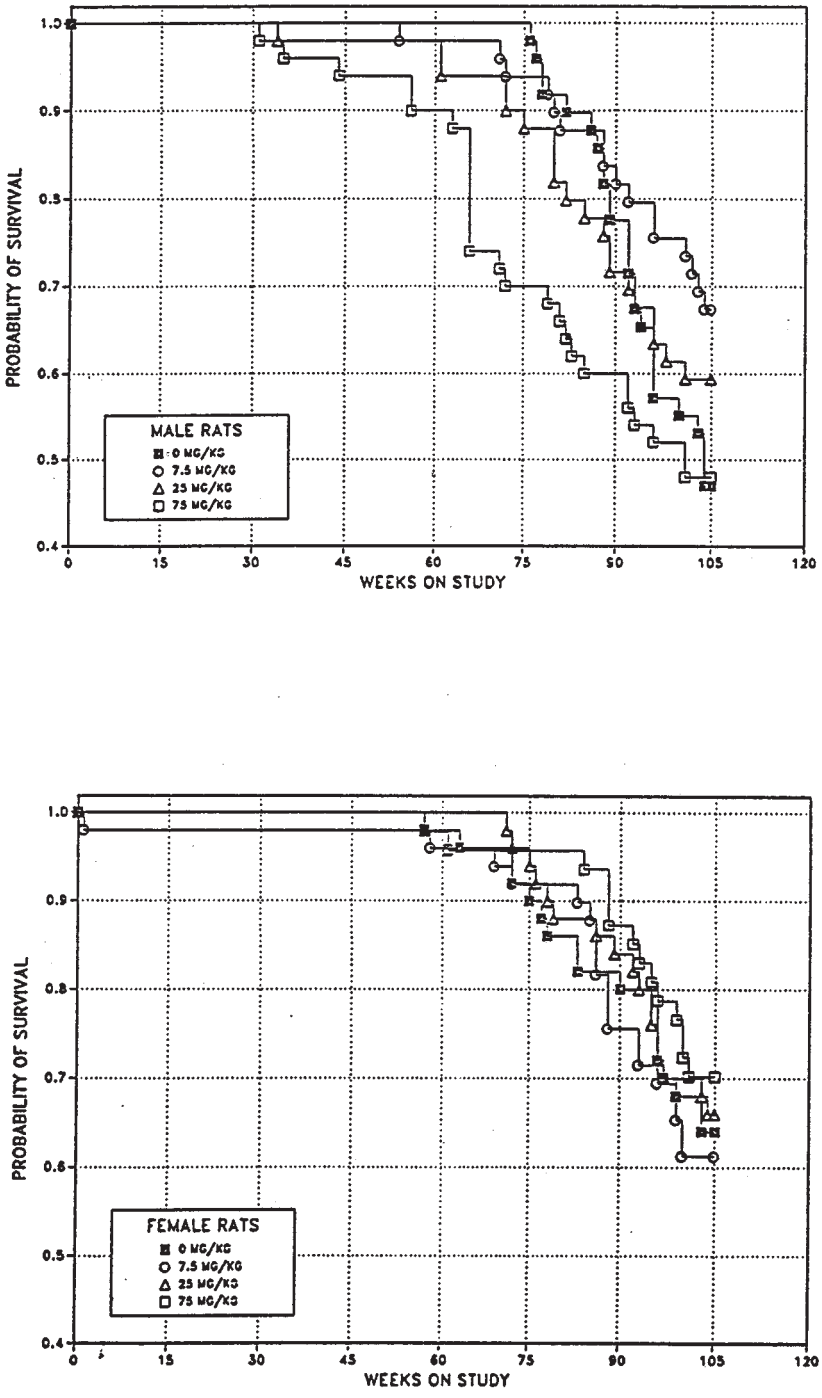


FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered Theophylline by Gavage for 2 Years

TABLE 10
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of Theophylline

Weeks on Study	Vehicle Control		7.5 mg/kg			25 mg/kg			75 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	145	50	145	100	50	144	100	50	143	99	50
2	198	50	195	99	50	196	99	50	191	97	50
3	222	50	217	98	50	217	98	50	211	95	50
4	243	50	237	97	50	235	97	50	227	94	50
5	262	50	252	96	50	255	97	50	249	95	50
6	277	50	267	97	50	266	96	50	262	95	50
7	291	50	282	97	50	278	96	50	273	94	50
8	300	50	290	97	50	284	95	50	282	94	50
9	310	50	299	97	50	291	94	50	287	93	50
10	320	50	309	97	50	300	94	50	296	93	50
11	330	50	319	97	50	311	94	50	306	93	50
12	338	50	325	96	50	315	93	50	309	92	50
13	344	50	331	96	50	320	93	50	315	92	50
16	358	50	345	97	50	329	92	50	329	92	50
20	388	50	365	94	50	354	91	50	348	90	50
24	397	50	366	92	50	354	89	50	347	87	50
28	426	50	392	92	50	373	88	50	372	87	50
32	437	50	402	92	50	387	88	50	381	87	49
36	445	50	404	91	49	388	87	49	383	86	48
40	454	50	409	90	49	393	87	49	393	87	48
44	463	50	412	89	49	398	86	49	400	86	47
48	470	50	418	89	49	407	87	49	411	87	47
52	477	50	422	88	49	409	86	49	406	85	47
56	480	49	425	88	48	413	86	49	412	86	45
60	482	49	424	88	48	414	86	49	412	86	45
64	485	49	420	87	48	410	85	47	413	85	44
68	489	49	421	86	48	416	85	47	415	85	37
72	491	49	416	85	46	418	85	45	411	84	35
76	479	48	407	85	46	414	87	43	410	86	35
80	481	45	405	84	44	408	85	40	404	84	34
84	472	44	398	84	43	403	85	39	403	85	31
88	467	40	394	84	43	407	87	37	401	86	30
92	464	35	395	85	39	411	89	34	404	87	28
96	459	28	389	85	37	411	90	31	391	85	26
100	456	27	381	84	37	410	90	30	387	85	26
Mean for weeks											
1-13	275		267	97		262	95		258	94	
14-52	432		394	91		379	88		377	87	
53-100	475		406	85		411	87		405	85	

TABLE 11
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of Theophylline

Weeks on Study	Vehicle Control		7.5 mg/kg			25 mg/kg			75 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	103	50	102	100	50	102	99	50	102	99	50
2	126	50	125	99	49	122	97	50	120	96	50
3	137	50	135	99	49	132	96	50	133	97	50
4	150	50	147	98	49	143	95	50	146	97	50
6	162	50	158	98	49	155	95	50	160	99	50
7	167	50	164	98	49	159	95	50	165	99	50
8	171	50	168	98	49	163	95	50	172	101	50
9	174	50	171	98	49	168	97	50	175	101	50
10	179	50	176	98	49	173	96	50	182	102	50
11	182	50	178	98	49	176	97	50	182	100	50
12	185	50	181	98	49	178	96	50	186	101	50
13	188	50	184	98	49	181	97	50	188	100	50
17	199	50	194	98	49	189	95	50	201	101	50
21	207	50	200	97	49	196	95	50	209	101	50
25	215	50	209	97	49	205	95	50	213	99	50
29	221	50	212	96	49	205	93	50	217	98	50
33	227	50	213	94	49	207	91	50	224	99	50
37	232	50	218	94	49	209	90	50	224	97	48
41	235	50	221	94	49	212	90	50	228	97	48
45	247	50	224	91	48	212	86	50	228	92	48
49	256	50	235	92	48	219	86	50	236	92	47
53	268	50	242	90	48	222	83	50	237	89	47
57	277	50	248	90	48	228	82	50	238	86	46
61	286	49	254	89	47	235	82	50	245	85	45
65	294	48	262	89	47	237	81	50	246	83	45
69	301	48	261	87	47	245	81	50	255	85	45
73	305	46	265	87	45	243	80	48	254	83	45
77	303	44	269	89	45	245	81	46	253	84	45
81	303	43	267	88	45	254	84	44	259	85	45
85	307	41	268	88	44	255	83	44	259	85	44
89	307	41	270	88	37	263	86	43	267	87	41
93	314	40	277	88	36	262	84	41	269	86	40
97	315	36	276	88	34	259	82	35	269	85	37
101	321	34	278	87	30	263	82	35	268	84	34
Mean for weeks											
1-13	160		157	98		154	96		159	99	
14-52	227		214	94		206	91		220	97	
53-101	300		264	88		247	82		255	85	

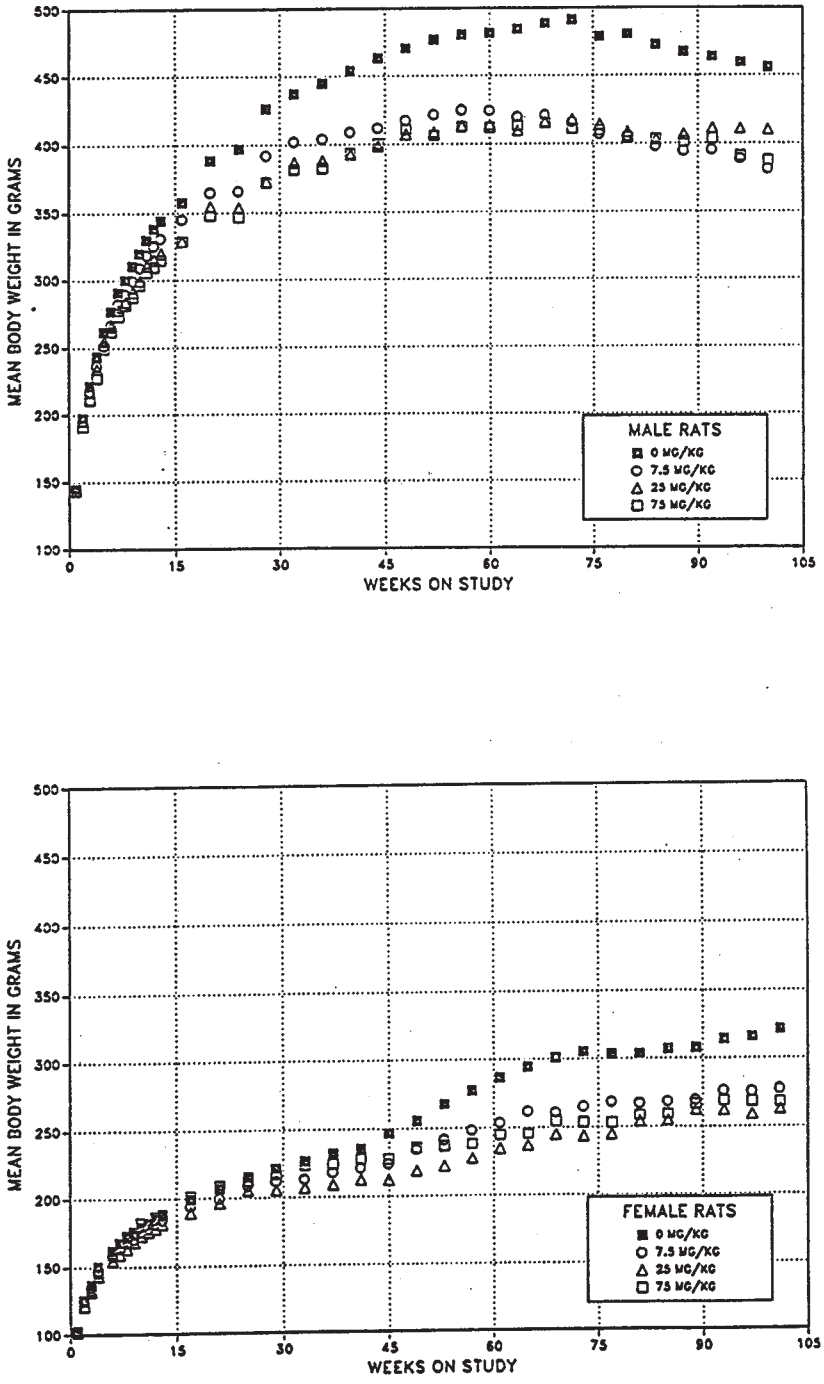


FIGURE 3
Growth Curves for Rats Administered Theophylline by Gavage for 2 Years

Pathology and Statistical Analyses

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

Mesenteric Arteries: In a special review of the mesentery and associated tissues from all 50 animals in each group, the incidence of chronic inflammation of the mesenteric arteries was significantly increased in male rats given 75 mg/kg (vehicle control, 2/50; 7.5 mg/kg, 2/50; 25 mg/kg, 3/50; 75 mg/kg, 15/50; Table A5). The periarteritis most frequently involved the medium to large arteries associated with the pancreas and, less often, with the mesenteric lymph nodes (Plates 7, 8, and 9). In general, the vascular lesions consistently involved the adventitia and, in more severe lesions, the media and intima. The adventitia was expanded by perivascular fibrosis with infiltrates of small macrophages mixed with low numbers of lymphocytes and degenerate cellular debris. A few macrophages contained cytoplasmic golden-brown pigment. Within affected media, smooth muscle cells were disordered and occasionally contained cytoplasmic vacuoles. Some severely affected arteries had combinations of adventitial and medial thickening, intense mononuclear cell and neutrophilic infiltrates, focal intimal and/or medial hemorrhage, and fibrinoid necrosis with small foci of mineralization.

Mononuclear Cell Leukemia: The incidences of mononuclear cell leukemia in 7.5 and 25 mg/kg males were significantly lower than that in controls (15/50, 5/50, 6/50, 6/50; Table A3). Incidences of mononuclear cell leukemia in dosed males were at the lower end of the range observed in historical controls from NTP 2-year corn oil gavage studies (Table A4). Because no dose relationship was noted and the incidences were within the historical control range for gavage studies, the lower incidences were not considered to be related to theophylline administration.

Mammary Gland: There were dose-related negative trends in the incidences of fibroadenoma and fibroadenoma or carcinoma (combined) in females, and the incidences in females dosed with 25 or 75 mg/kg were significantly lower than those in the control group (Tables 12 and B3). The incidences of fibroadenoma and fibroadenoma or carcinoma (combined) in these groups were at the lower end of the range of incidences found in historical vehicle controls (Table B4).

TABLE 12
Incidences of Mammary Gland Neoplasms in Female Rats in the 2-Year Gavage Study of Theophylline

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Fibroadenoma[a]				
Overall rate[b]	22/50 (44%)	19/50 (38%)	12/50 (24%)	12/50 (24%)
Adjusted rate[c]	54.4%	53.6%	32.2%	31.6%
Terminal rate[d]	14/32 (44%)	14/30 (47%)	9/33 (27%)	8/33 (24%)
First incidence (days)	498	614	498	393
Logistic regression test[e]	P=0.023N	P=0.391N	P=0.025N	P=0.030N
Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Fibroadenoma or Carcinoma[f]				
Overall rate	23/50 (46%)	20/50 (40%)	12/50 (24%)	12/50 (24%)
Adjusted rate	57.0%	55.0%	32.2%	31.6%
Terminal rate	15/32 (47%)	14/30 (47%)	9/33 (27%)	8/33 (24%)
First incidence (days)	498	614	498	393
Logistic regression test	P=0.013N	P=0.394N	P=0.015N	P=0.019N

[a] Historical incidence for NTP gavage studies with corn oil control groups (mean ± standard deviation): 349/971 (35.9% ± 9.7%); range 24%-56%
[b] Number of animals with neoplasm per number of animals necropsied
[c] Kaplan-Meier estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence in animals surviving until the end of the study
[e] In the vehicle control column are the P values associated with the trend test. In the dosed group columns are the P values corresponding to the pairwise comparisons between the vehicle control and that dosed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in a dosed group is indicated by **N**.
[f] Historical incidence: 363/971 (37.4% ± 9.5%); range 28%-56%

MICE

16-DAY FEED STUDY

All mice survived until the end of the study (Table 13). Final mean body weights of 4,000 and 8,000 ppm females and mean body weight gains of 2,000, 4,000, and 8,000 ppm females were significantly greater than those of the controls. Feed consumption by exposed groups was similar to that by the controls, except that by the 8,000 ppm males; these males consumed approximately 40% the amount of feed consumed by the control group. Dietary levels of 500, 1,000, 2,000, 4,000, or 8,000 ppm resulted in approximate daily doses of 250, 475, 950, 1,800, or 2,000 mg/kg to male mice and 300, 450, 1,225, 2,000, or 4,375 mg/kg to female mice. There were no clinical findings attributed to theophylline exposure.

The hematocrit values of 4,000 ppm males and hemoglobin concentrations of 4,000 and 8,000 ppm males were significantly increased compared to the control group (Table G6).

The absolute and relative kidney weights of males receiving 8,000 ppm were significantly less than those of the controls (Table F6).

Necropsy revealed no treatment-related gross lesions. Histopathologic examinations were not performed due to the absence of mortality and significant exposure-related gross lesions.

TABLE 13
Survival, Body Weights, and Feed Consumption of Mice in the 16-Day Feed Study of Theophylline

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2[d]
Male							
0	5/5	20.2 ± 0.2	23.2 ± 0.4	3.0 ± 0.5		367.0	467.8
500	5/5	20.6 ± 0.2	24.0 ± 0.5	3.4 ± 0.5	103	541.6	479.4
1,000	5/5	20.8 ± 0.2	23.8 ± 0.5	3.0 ± 0.3	103	475.8	497.9
2,000	5/5	19.4 ± 0.2	23.2 ± 0.7	3.8 ± 0.9	100	535.5	423.5
4,000	5/5	20.2 ± 0.4	23.2 ± 0.4	3.0 ± 0.3	100	433.9	472.0
8,000	5/5	20.2 ± 0.4	22.6 ± 0.7	2.4 ± 0.5	97	276.3	223.2
Female							
0	5/5	15.8 ± 0.4	17.4 ± 0.6	1.6 ± 0.4		462.1	614.0
500	5/5	16.2 ± 0.2	18.4 ± 0.4	2.2 ± 0.2	106	529.4	656.7
1,000	5/5	15.0 ± 0.3	17.6 ± 0.5	2.6 ± 0.4	101	492.9	384.3
2,000	5/5	15.0 ± 0.0	18.6 ± 0.4	3.6 ± 0.4**	107	625.4	594.8
4,000	5/5	15.8 ± 0.4	19.2 ± 0.6*	3.4 ± 0.4**	110	574.7	425.0
8,000	5/5	15.6 ± 0.2	19.6 ± 0.4**	4.0 ± 0.3**	113	556.7	533.5

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per kilogram animal weight per day.
[d] Eight days in week 2 for females

16-DAY GAVAGE STUDY

Three males and all females receiving 400 mg theophylline/kg body weight once daily died on study day 1 (Table 14). There were no significant differences in final mean body weights or body weight gains between groups exposed once daily and controls or groups exposed twice daily (Tables 14 and 15). Clinical findings of toxicity observed during this study included squinting or partial squinting, distended testes, sternal recumbency, sluggishness, white discharge from eyes, hunched posture, intermittent convulsion, rapid respiration, and hind-limb paralysis.

There were no significant differences in hematology values (Tables G7 and G8) and no biologically significant differences in organ weights between control and dosed mice (Tables F7 and F8).

TABLE 14
Survival and Body Weights of Mice in the 16-Day Gavage Study of Theophylline: Comparison of Groups Receiving Once-Daily Administration

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	23.0 ± 0.7	25.0 ± 0.6	2.0 ± 0.6	
25	5/5	22.6 ± 0.8	24.6 ± 0.8	2.0 ± 0.6	98
50	5/5	22.6 ± 0.5	24.6 ± 0.5	2.0 ± 0.3	98
100	5/5	22.6 ± 0.4	24.0 ± 0.6	1.4 ± 0.4	96
200	5/5	23.4 ± 0.4	26.0 ± 0.6	2.6 ± 0.4	104
400	2/5[c]	23.6 ± 0.4	26.0 ± 0.0	3.0 ± 0.0	104
Female					
0	5/5	18.4 ± 0.2	21.0 ± 0.6	2.6 ± 0.4	
25	5/5	19.2 ± 0.2	22.0 ± 0.6	2.8 ± 0.6	105
50	5/5	19.0 ± 0.5	20.8 ± 0.6	1.8 ± 0.4	99
100	5/5	17.8 ± 0.5	19.6 ± 0.5	1.8 ± 0.6	93
200	5/5	19.0 ± 0.5	22.0 ± 0.5	3.0 ± 0.3	105
400	0/5[c]	20.6 ± 0.5**	—	—	

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. No final mean body weights were calculated for groups with 100% mortality.
[c] All deaths occurred on day 1.

TABLE 15
Survival and Body Weights of Mice in the 16-Day Gavage Study of Theophylline: Comparisons of Once-Daily to Twice-Daily Administration

Dose (mg/kg)		Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Twice-Daily Group (%)
			Initial	Final	Change	
Male						
Low-Dose Comparison						
12.5	twice daily	5/5	22.8 ± 0.4	24.4 ± 0.4	1.6 ± 0.2	
25	once daily	5/5	22.6 ± 0.8	24.6 ± 0.8	2.0 ± 0.6	101
Mid-Dose Comparison						
50	twice daily	5/5	22.6 ± 0.5	25.0 ± 0.6	2.4 ± 0.5	
100	once daily	5/5	22.6 ± 0.4	24.0 ± 0.6	1.4 ± 0.4	96
High-Dose Comparison						
200	twice daily	5/5	23.6 ± 0.5	25.6 ± 0.5	2.0 ± 0.0	
400	once daily	2/5[c]	23.6 ± 0.4	26.0 ± 0.0	3.0 ± 0.0	102
Female						
Low-Dose Comparison						
12.5	twice daily	5/5	19.4 ± 0.6	21.2 ± 0.6	1.8 ± 0.5	
25	once daily	5/5	19.2 ± 0.2	22.0 ± 0.6	2.8 ± 0.6	104
Mid-Dose Comparison						
50	twice daily	5/5	19.4 ± 0.5	20.6 ± 0.2	1.2 ± 0.4	
100	once daily	5/5	17.8 ± 0.5	19.6 ± 0.5	1.8 ± 0.6	95
High-Dose Comparison						
200	twice daily	5/5	—	—	—	
400	once daily	0/5[c]	—	—	—	

[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. No final mean body weights were calculated for groups with 100% mortality. Differences between the once-daily group and twice-daily group were not significant by a *t*-test.
[c] All deaths occurred on day 1.

Histopathologic examination revealed mild lung congestion in males and females (males: vehicle control, 0/5; 200 mg/kg once daily, 1/5; 200 mg/kg twice daily, 0/5; 400 mg/kg once daily, 0/5; females: 0/5, 0/5, 0/5, 3/5). The lung changes were considered nonspecific agonal changes accompanying death resulting from theophylline administration.

14-WEEK FEED STUDY

All mice survived until the end of the study (Table 16). The final mean body weights and body weight gains of all exposed groups were significantly less than those of the controls. Feed consumption by exposed groups was similar to that by the control groups. Dietary levels of 1,000, 2,000, or 4,000 ppm resulted in approximate daily doses of 175, 400, or 800 mg theophylline/kg body weight to males and 225, 425, or 850 mg/kg to females. There were no clinical findings related to theophylline exposure.

TABLE 16
Survival, Body Weights, and Feed Consumption of Mice in the 14-Week Feed Study of Theophylline

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 2	Week 13
Male							
0	10/10	22.4 ± 0.3	34.3 ± 0.7	12.0 ± 0.6		197.4	128.3
1,000	10/10	23.5 ± 0.4*	29.8 ± 0.5**	6.2 ± 0.6**	87	235.6	147.7
2,000	10/10	22.9 ± 0.3	29.2 ± 0.4**	6.3 ± 0.3**	85	238.5	154.1
4,000	10/10	23.2 ± 0.3	28.8 ± 0.3**	5.5 ± 0.4**	84	187.8	177.1
Female							
0	10/10	18.4 ± 0.2	29.3 ± 0.7	10.9 ± 0.7		261.3	181.5
1,000	10/10	18.5 ± 0.3	26.8 ± 0.5**	8.4 ± 0.3**	92	278.1	190.3
2,000	10/10	18.1 ± 0.4	27.1 ± 0.4*	9.0 ± 0.6*	93	238.1	177.1
4,000	10/10	18.7 ± 0.2	27.4 ± 0.4*	8.7 ± 0.3*	93	204.0	183.2

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett' test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per kilogram animal weight per day.

Leukocyte, segmented neutrophil, and lymphocyte counts of 4,000 ppm males were significantly greater than those of the controls (Table G9). Leukocyte and segmented neutrophil counts were significantly greater in groups of females exposed to 2,000 or 4,000 ppm than those in the control group. There were no biologically significant differences in sperm morphology or vaginal cytology parameters between control and exposed mice (Table H3).

The absolute thymus weight of 1,000 ppm females and the absolute and relative thymus weights of 2,000 and 4,000 ppm females were significantly less than those of control females (Table F9). No significant exposure-related lesions were observed at necropsy. The incidences of hepatocyte glycogen depletion in exposed male and female mice were greater than those in the controls (Table 17). Glycogen depletion (confirmed by PAS staining) was characterized by the absence of poorly delineated, irregular vacuoles common in the cytoplasm of hepatocytes. The glycogen depletion was most often centrilobular in distribution, but occasionally involved entire lobules. Hepatocyte glycogen depletion is considered to be the result of lower body weights due to the administration of theophylline.

TABLE 17
Incidences of Nonneoplastic Lesions of the Liver in Mice in the 14-Week Feed Study of Theophylline

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Male				
Number Examined Microscopically	10	10	10	10
Glycogen Depletion[a]	3 (2.6)[b]	10** (1.8)	9** (2.2)	10** (2.6)
Female				
Number Examined Microscopically	10	10	10	10
Glycogen Depletion	0	6** (2.2)	9** (2.0)	10** (2.0)

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test
[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

14-WEEK GAVAGE STUDY

Three males and all females receiving 300 mg/kg, one male receiving 75 mg/kg, and one control female died before the end of the study (Table 18). The final mean body weights and body weight gains of 150 and 300 mg/kg males were significantly less than those of the control group. There were no clinical findings attributed to theophylline treatment.

The mean cell volume and mean cell hemoglobin of 300 mg/kg males were significantly greater than those of the controls (Table G10). There were no biologically significant differences in sperm morphology or vaginal cytology parameters between controls and dosed mice (Table H4). No biologically significant organ weight differences were observed (Table F10). Histopathologic examination revealed significantly greater incidences of hepatocyte glycogen depletion in 75 and 150 mg/kg females than that in the control group (Table 19). The livers of female mice that died or were killed moribund during the study were not examined for glycogen depletion. Glycogen depletion uniformly involved the entire hepatic lobule, but occasionally was more pronounced in periportal areas. Minimal to moderate lymphoid depletion was observed in the thymus and spleen of 300 mg/kg males and was considered to be related to stress associated with theophylline administration. The incidences of lung congestion were increased in 300 mg/kg males and females (significantly in females). Lung congestion was considered to be a nonspecific change accompanying agonal death and not a direct effect of theophylline treatment.

TABLE 18
Survival and Body Weights of Mice in the 14-Week Gavage Study of Theophylline

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.3 ± 0.7	38.0 ± 1.3	14.7 ± 1.0	
75	9/10[c]	23.9 ± 0.3	36.3 ± 0.8	12.5 ± 0.7	95
150	10/10	23.3 ± 0.9	34.0 ± 0.5**	10.7 ± 1.0**	89
300	7/10[d]	24.6 ± 0.4	32.8 ± 0.7**	7.8 ± 0.5**	86
Female					
0	9/10[e]	18.5 ± 0.7	29.6 ± 0.7	11.0 ± 0.7	
75	10/10	17.5 ± 0.7	28.1 ± 0.4	10.6 ± 0.6	95
150	10/10	18.9 ± 0.3	28.5 ± 0.7	9.6 ± 0.6	96
300	0/10[f]	18.9 ± 0.3	30.7[g]	11.6	104

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett' test
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. No standard errors were calculated for groups with high mortality.
[c] Week of death: 14
[d] Week of death: 10, 11, 13
[e] Week of death: 6 (accidental death)
[f] Week of death: 1, 1, 1, 1, 1, 1, 9, 9, 11, 14
[g] One female died on day 96, at the end of dosing.

TABLE 19
Incidences of Selected Nonneoplastic Lesions in Mice in the 14-Week Gavage Study of Theophylline

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Male				
Lung[a]	10	—[c]	10	10
Congestion[b]	0		0	3 (2.7)[d]
Spleen	10	—	10	10
Lymphoid Follicle, Depletion	0		0	2 (2.0)
Thymus	10	—	10	10
Depletion	0		0	3 (1.3)
Female				
Liver	9[e]	10	10	—[e]
Hepatocyte Glycogen Depletion	1 (2.0)	10** (2.5)	7**	(2.6) —
Lung	10	—	10	10
Congestion	0		0	10** (3.0)

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test
[a] Number of animals with organ/tissue examined microscopically
[b] Number of animals with lesion
[c] Tissue not examined at this dose level
[d] Average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[e] One control female and all 300 mg/kg females died before the end of the study; the livers of these mice were not examined for glycogen depletion.

Dose Selection Rationale: Based on survival rates of mice receiving 300 mg/kg for 14 weeks, males seemed to be more resistant than females to the toxic effects of theophylline. Based on survival and histopathologic changes (lymphoid depletion in males and hepatocyte glycogen depletion in females) observed in the 14-week study, the doses selected for the 2-year study were 0, 15, 50, and 150 mg/kg for male mice and 0, 7.5, 25, and 75 mg/kg for female mice. The gavage route of administration was selected to mimic human therapeutic use of theophylline.

2-YEAR GAVAGE STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 20 and in the Kaplan-Meier survival curves (Figure 4). Survival of males receiving 150 mg/kg was significantly less than that of the controls.

Body Weights and Clinical Findings

The mean body weights of 150 mg/kg males and of 25 and 75 mg/kg females were significantly less than those of controls throughout most of the study (Figure 5 and Tables 21 and 22). Hyperactivity was observed in four 75 mg/kg females.

TABLE 20
Survival of Mice in the 2-Year Gavage Study of Theophylline

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Male				
Animals initially in study	50	50	50	50
Moribund	9	9	2	9
Natural deaths	5	6	4	15
Animals surviving to study termination	36	35	44	26
Percent probability of survival at end of study[a]	72	70	88	52
Mean survival (days)[b]	701	683	714	544
Survival analysis[c]	P=0.001	P=0.838	P=0.090N	P=0.014
	Vehicle Control	**7.5 mg/kg**	**25 mg/kg**	**75 mg/kg**
Female				
Animals initially in study	50	50	50	50
Moribund	7	4	11	9
Natural deaths	6	9	5	8
Animals surviving to study termination	37	37	34	33
Percent probability of survival at end of study	74	74	68	66
Mean survival (days)	696	699	705	673
Survival analysis	P=0.407	P=1.000N	P=0.851	P=0.537

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed group columns. A lower mortality in a dosed group is indicated by **N**.

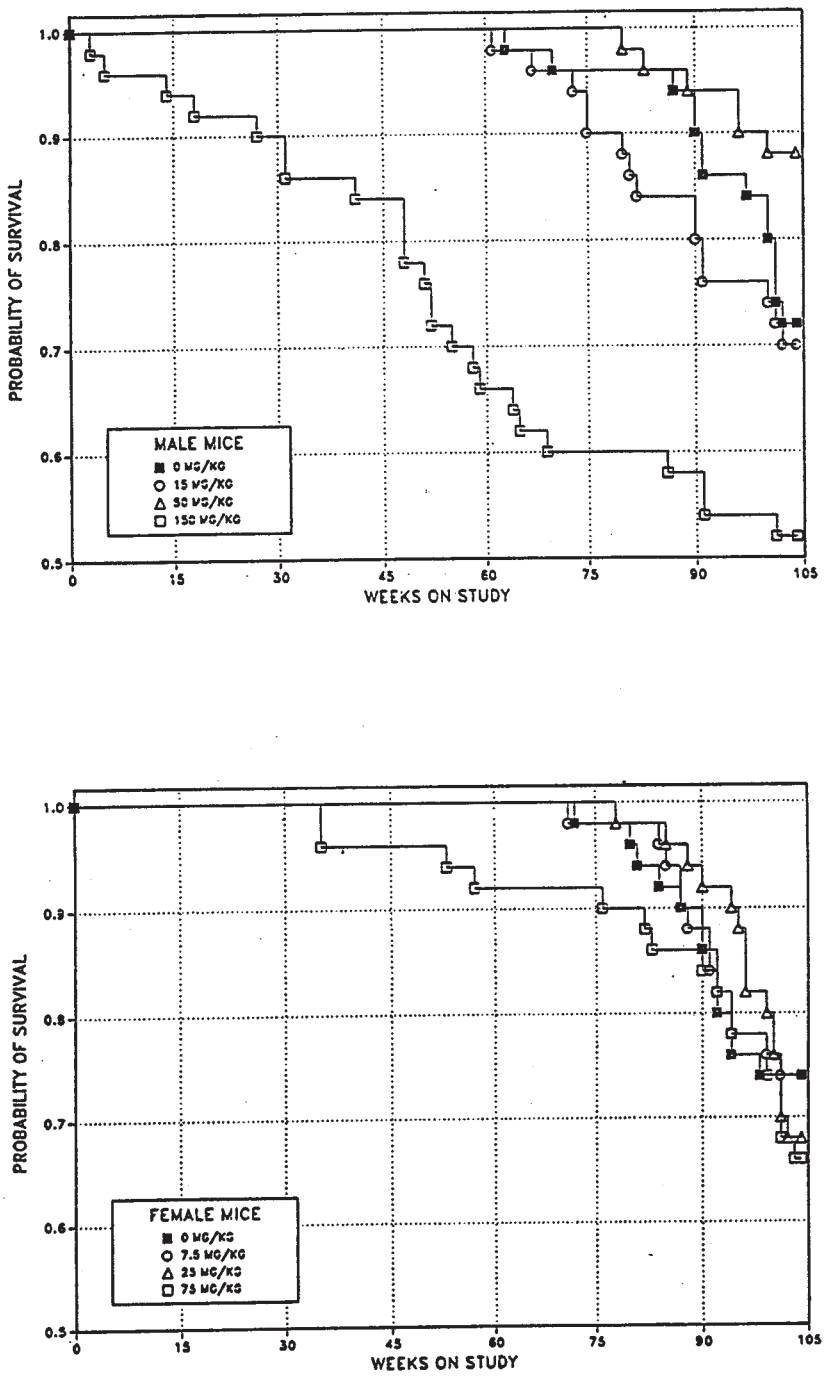


FIGURE 4
Kaplan-Meier Survival Curves for Mice Administered Theophylline by Gavage for 2 Years

TABLE 21
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of Theophylline

Weeks on Study	Vehicle Control		15 mg/kg			50 mg/kg			150 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.5	50	22.8	97	50	23.1	98	50	23.0	98	50
2	25.3	50	24.9	98	50	24.9	98	50	25.2	100	50
3	26.4	50	26.0	99	50	25.9	98	50	26.2	99	49
4	27.4	50	27.2	99	50	27.2	99	50	27.5	100	49
5	28.6	50	28.4	99	50	28.0	98	50	28.1	98	49
6	29.4	50	28.9	98	50	28.5	97	50	28.5	97	48
7	31.7	50	31.0	98	50	31.0	98	50	30.8	97	48
8	32.1	50	31.7	99	50	31.3	98	50	30.8	96	48
9	33.6	50	33.2	99	50	32.9	98	50	32.2	96	48
10	34.8	50	34.5	99	50	34.6	99	50	33.2	95	48
11	35.5	50	34.9	98	50	34.5	97	50	33.6	95	48
12	36.2	50	35.7	99	50	35.1	97	50	32.8	91	48
13	37.3	50	37.0	99	50	36.1	97	50	33.7	90	48
14	37.7	50	37.1	98	50	36.2	96	50	33.6	89	48
15	38.8	50	38.2	99	50	37.8	97	50	35.3	91	47
17	41.3	50	40.4	98	50	39.9	97	50	36.9	89	47
21	44.1	50	43.0	98	50	41.7	95	50	37.7	86	46
25	46.4	50	45.4	98	50	43.2	93	50	38.2	82	46
29	47.5	50	46.6	98	50	44.8	94	50	40.1	84	45
33	48.3	50	47.3	98	50	45.4	94	50	40.0	83	43
37	49.4	50	48.4	98	50	46.0	93	50	40.5	82	43
41	50.6	50	49.4	98	50	47.1	93	50	40.9	81	42
45	51.3	50	50.4	98	50	48.2	94	50	42.1	82	42
49	52.1	50	50.8	98	50	48.8	94	50	42.4	81	39
53	52.0	50	51.4	99	50	49.6	95	50	41.8	80	36
57	52.3	50	51.9	99	50	49.9	95	50	41.9	80	35
61	52.5	50	52.5	100	49	51.1	97	50	43.5	83	33
65	53.2	49	52.6	99	49	51.0	96	50	44.1	83	31
69	53.6	49	52.4	98	48	51.0	95	50	43.6	81	31
73	52.9	48	51.0	96	48	50.5	96	50	43.9	83	30
77	52.9	48	52.6	99	45	50.8	96	50	43.8	83	30
81	51.2	48	51.2	100	43	49.4	97	49	41.5	81	30
85	51.2	48	52.5	103	42	51.2	100	48	44.1	86	30
89	50.9	47	51.1	100	42	51.2	101	48	42.9	84	29
93	50.6	43	51.5	102	38	50.1	99	47	41.1	81	27
97	48.5	43	50.3	104	38	49.9	103	45	41.4	85	27
101	46.4	38	49.7	107	37	48.7	105	44	40.8	88	26
Mean for weeks											
1-13	30.9		30.5	99		30.2	98		29.7	96	
14-52	46.1		45.2	98		43.6	95		38.9	84	
53-101	51.4		51.6	100		50.3	98		42.6	83	

TABLE 22
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of Theophylline

Weeks on Study	Vehicle Control		7.5 mg/kg			25 mg/kg			75 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.2	50	18.9	98	50	19.0	99	50	19.1	100	50
2	20.7	50	20.5	99	50	20.6	100	50	20.8	101	50
3	22.0	50	21.9	100	50	21.8	99	50	22.2	101	50
4	22.8	50	22.3	98	50	22.5	99	50	22.4	98	50
5	23.6	50	23.3	99	50	23.1	98	50	23.2	98	50
6	24.4	50	24.1	99	50	23.9	98	50	23.9	98	50
7	26.7	50	26.5	99	50	26.1	98	50	26.4	99	50
8	27.1	50	26.8	99	50	26.9	99	50	27.0	100	50
9	27.7	50	27.4	99	50	27.2	98	50	27.4	99	50
10	28.8	50	28.6	99	50	28.5	99	50	27.9	97	50
11	30.1	50	29.4	98	50	29.3	97	50	29.3	97	50
12	30.4	50	30.2	99	50	30.3	100	50	30.0	99	50
13	30.6	50	30.3	99	50	30.2	99	50	29.8	97	50
14	31.8	50	31.8	100	50	31.7	100	50	31.1	98	50
15	33.8	50	33.7	100	50	33.5	99	50	32.5	96	50
17	35.0	50	34.8	99	50	35.0	100	50	33.9	97	50
21	38.0	50	37.2	98	50	36.8	97	50	35.4	93	50
25	40.5	50	39.3	97	50	38.9	96	50	37.1	92	50
29	43.8	50	42.6	97	50	41.2	94	50	40.0	91	50
33	44.4	50	43.8	99	50	42.7	96	50	40.7	92	50
37	46.7	50	45.5	97	50	44.1	94	50	42.8	92	48
41	47.3	50	46.6	99	50	44.5	94	50	42.9	91	48
45	49.0	50	47.8	98	50	45.8	94	50	43.9	90	48
49	50.5	50	49.0	97	50	46.5	92	50	44.6	88	48
53	51.4	50	50.0	97	50	47.1	92	50	45.5	89	47
57	52.6	50	51.3	98	50	48.7	93	50	46.9	89	46
61	54.1	50	53.0	98	50	49.2	91	50	47.4	88	46
65	55.5	50	54.5	98	50	50.2	91	50	48.4	87	46
69	56.6	50	56.0	99	50	51.1	90	50	49.1	87	46
73	56.7	49	55.6	98	49	50.5	89	50	49.4	87	46
77	56.3	49	55.6	99	49	50.4	90	50	48.4	86	45
81	56.3	47	55.3	98	49	51.3	91	49	48.5	86	45
85	57.2	46	55.1	96	47	51.7	90	49	49.3	86	43
89	56.8	45	55.6	98	44	51.6	91	47	48.9	86	43
93	57.6	40	55.6	97	41	51.5	89	46	47.9	83	41
97	56.6	38	54.4	96	39	50.3	89	41	46.5	82	39
101	55.2	37	54.1	98	37	49.6	90	35	45.5	82	34
Mean for weeks											
1-13	25.7		25.4	99		25.3	98		25.3	98	
14-52	41.9		41.1	98		40.1	96		38.6	92	
53-101	55.6		54.3	98		50.2	90		47.8	86	

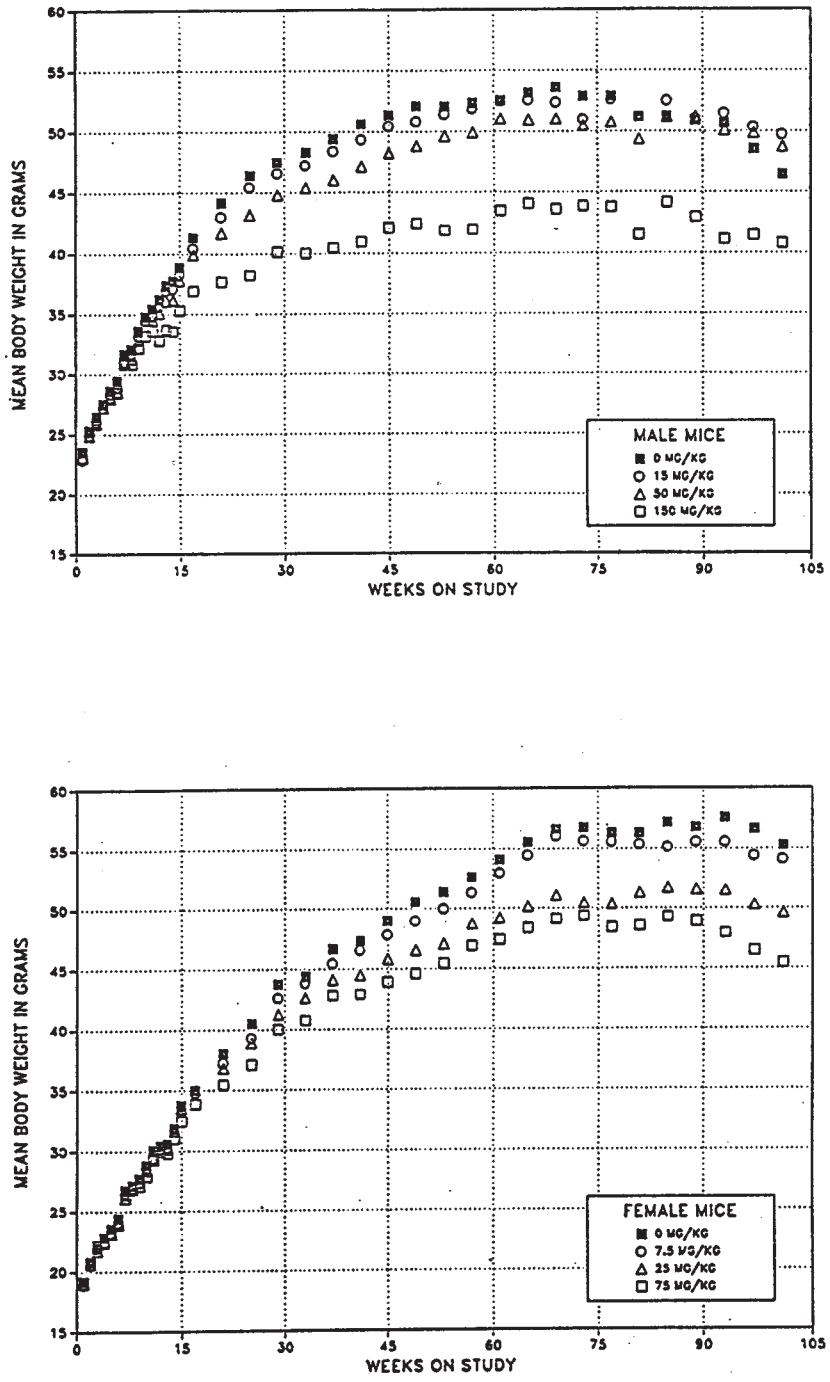


FIGURE 5
Growth Curves for Mice Administered Theophylline by Gavage for 2 Years

Pathology and Statistical Analyses

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

Kidney: A few males receiving 150 mg/kg had renal tubule degeneration (7/50) and renal tubule dilatation (4/50) (Table C5). These lesions did not occur in control males or the lower dose groups, but did occur in control and dosed females (Table D5) that died spontaneously or were killed moribund, and were therefore considered to be related to agonal death rather than to a direct effect of theophylline administration. Renal tubule degeneration involved tubules in the outer stripe of the medulla. Epithelial cells of affected tubules had cytoplasmic hypereosinophilia and small dark (pyknotic) nuclei. Some cells were sloughed into the tubular lumen and occasional granular casts composed of cellular debris were observed. In some instances, these changes were associated with renal tubule dilatation of the distal convoluted tubules. Males receiving 150 mg/kg had a lower incidence of nephropathy than did the controls (vehicle control, 46/50; 15 mg/kg, 46/49; 50 mg/kg, 45/50; 150 mg/kg, 29/50). The lower incidence was attributed to poor survival in this group.

Spleen and Thymus: The incidences of cellular depletion in the spleen and necrosis in the thymus of 150 mg/kg males were significantly greater than those in the controls (Tables 23 and C5). Grossly, the affected spleens were smaller than those of the controls, and microscopically, they had decreased cellularity in the red and/or white pulps.

Thymic necrosis was characterized by pyknotic and karyorrhectic nuclei in cells of the cortical and medullary regions. The splenic and thymic alterations were observed in male mice that died spontaneously or were killed moribund and had markedly lower final mean body weights and body weight gains than the controls. The histologic alterations in these tissues were attributed to lower body weights and/or stress, rather than to a direct toxic effect of theophylline administration.

TABLE 23
Incidences of Nonneoplastic Lesions of the Spleen and Thymus in Mice in the 2-Year Gavage Study of Theophylline

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Male				
Spleen[a]	50	49	50	49
Cellular Depletion[b]	1 (1.0)[c]	0	0	12** (2.6)
Thymus	43	42	44	46
Necrosis	0	1 (3.0)	1 (3.0)	11** (2.6)
	Vehicle Control	**7.5 mg/kg**	**25 mg/kg**	**75 mg/kg**
Female				
Spleen	50	50	49	48
Cellular Depletion	0	0	0	2 (2.5)
Thymus	47	44	49	47
Necrosis	1 (3.0)	0	1 (3.0)	2 (2.0)

** Significantly different ($P \leq 0.01$) from the control group by the life table test
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Thyroid Gland: There was a dose-dependent increase in the incidences of thyroid gland cystic degeneration; males receiving 150 mg/kg had a significantly greater incidence of this lesion than did the controls (males: 6/50, 11/50, 10/50, 13/50; females: vehicle control, 12/50; 7.5 mg/kg, 13/50; 25 mg/kg, 10/50; 75 mg/kg, 15/50; Tables C5 and D5). This lesion was characterized by focal groups of a few variably dilated follicles that were lined by flattened epithelial cells and contained pale eosinophilic colloid with or without sloughed epithelial cells. Cystic degeneration of the follicles is a com-monly observed change in the thyroid glands of aging $B6C3F_1$ mice. In the present study, the significance of the increased incidences in the dosed mice is uncertain, but they are not considered to be related to theophylline administration.

Liver: In males and females, there were dose-dependent decreased incidences of hepatocellular adenoma and of the combined incidences of hepatocellular adenoma or carcinoma (Tables 24, C3, and D3). Males receiving 50 or 150 mg/kg and females receiving 7.5, 25, or 75 mg/kg had significantly lower incidences of hepatocellular adenoma than did the controls. Males receiving 150 mg/kg also had a significantly lower incidence of hepatocellular carcinoma than the controls. Additionally, 150 mg/kg males had significantly lower incidences of eosinophilic focus, chronic inflammation, cytoplasmic vacuolization, and hepatocyte karyomegaly than the controls.

The lower incidence of hepatocellular neoplasms in dosed mice may have been due in part to unusually high incidences of hepatocellular neoplasms in the controls. Compared to historical controls for 2-year corn oil gavage studies, vehicle control males and 15 mg/kg males had incidences of hepatocellular adenoma in the upper range of the historical controls and the incidences of hepatocellular carcinoma exceeded the incidences in the historical controls (Tables 24 and C4). In vehicle control females, the incidence of hepatocellular adenoma exceeded the incidences in the historical control range for gavage studies (Tables 24 and D4). In 150 mg/kg males, the

incidences of hepatocellular adenoma and carcinoma were below the lower limits of the historical control ranges.

The reduced survival and earlier deaths (the mean day of natural death was 348 for 150 mg/kg males and 639 for vehicle control males) and lower body weights may have contributed to the decreased incidence of hepatocellular neoplasms in the 150 mg/kg male mice.

Relatively high incidences of chronic inflammation and hepatocytic karyomegaly occurred in all groups of male mice except the 150 mg/kg group (Table 24), and both lesions usually occurred in the same livers. Chronic inflammation was characterized by oval cell hyperplasia, minimal to mild mononuclear inflammatory cell infiltrates, and, in more severe lesions, nodular regenerative hepatocellular hyperplasia. These changes were generally mild to moderate in severity, and were observed throughout the liver (usually not within proliferative lesions), but were most pronounced in the portal regions. Similar but less severe lesions were observed in a few females. Liver sections from four male mice with these liver lesions were examined and found positive for bacterial organisms consistent with *Helicobacter* when examined using Steiner's modification of the Warthin-Starry silver stain.

TABLE 24
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Theophylline

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Chronic Inflammation[a]	24 (2.2)[b]	25 (2.0)	16 (2.1)	3** (1.7)
Eosinophilic Focus	6 (2.2)	6 (2.3)	9 (2.7)	0*
Cytoplasmic Vacuolization	10 (2.1)	11 (1.7)	6 (1.8)	1* (2.0)
Hepatocyte Karyomegaly	15 (2.3)	14 (2.4)	12 (2.3)	2** (2.5)
Hepatocellular Adenoma[c]				
Overall rate[d]	21/50 (42%)	18/50 (36%)	12/50 (24%)	2/50 (4%)
Adjusted rate[e]	52.1%	48.1%	26.6%	7.7%
Terminal rate[f]	17/36 (47%)	16/35 (46%)	11/44 (25%)	2/26 (8%)
First incidence (days)	605	521	668	725 (T)
Logistic regression test[g]	P < 0.001N	P=0.447N	P=0.030N	P < 0.001N
Hepatocellular Carcinoma[h]				
Overall rate	19/50 (38%)	14/50 (28%)	12/50 (24%)	2/50 (4%)
Adjusted rate	42.0%	32.2%	26.0%	7.2%
Terminal rate	11/36 (31%)	7/35 (20%)	10/44 (23%)	1/26 (4%)
First incidence (days)	485	464	556	636
Logistic regression test	P=0.002N	P=0.129N	P=0.137N	P=0.001N
Hepatocellular Adenoma or Carcinoma[i]				
Overall rate	34/50 (68%)	27/50 (54%)	22/50 (44%)	4/50 (8%)
Adjusted rate	72.1%	62.2%	46.7%	14.6%
Terminal rate	23/36 (64%)	19/35 (54%)	19/44 (43%)	3/26 (12%)
First incidence (days)	485	464	556	636
Logistic regression test	P < 0.001N	P=0.113N	P=0.016N	P < 0.001N

(continued)

TABLE 24
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Theophylline (continued)

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Female				
Number Examined Microscopically	50	50	50	50
Chronic Inflammation	0	3 (1.0)	5* (1.6)	2 (1.5)
Cytoplasmic Vacuolization	6 (2.0)	8 (2.1)	5 (2.0)	2 (1.5)
Eosinophilic Focus	6 (2.2)	7 (1.9)	5 (2.4)	3 (2.3)
Hepatocyte Karyomegaly	0	0	1 (2.0)	0
Hepatocellular Adenoma[j]				
Overall rate	20/50 (40%)	11/50 (22%)	12/50 (24%)	3/50 (6%)
Adjusted rate	48.3%	29.7%	34.0%	9.1%
Terminal rate	16/37 (43%)	11/37 (30%)	11/34 (32%)	3/33 (9%)
First incidence (days)	624	725 (T)	669	725 (T)
Logistic regression test	P < 0.001N	P=0.035N	P=0.049N	P < 0.001N
Hepatocellular Carcinoma				
Overall rate	11/50 (22%)	5/50 (10%)	6/50 (12%)	5/50 (10%)
Adjusted rate	26.0%	11.8%	15.3%	14.6%
Terminal rate	7/37 (19%)	2/37 (5%)	3/34 (9%)	4/33 (12%)
First incidence (days)	501	591	667	704
Logistic regression test	P=0.156N	P=0.090N	P=0.171N	P=0.092N
Hepatocellular Adenoma or Carcinoma[k]				
Overall rate	29/50 (58%)	14/50 (28%)	18/50 (36%)	8/50 (16%)
Adjusted rate	64.1%	34.5%	46.7%	23.4%
Terminal rate	21/37 (57%)	11/37 (30%)	14/34 (41%)	7/33 (21%)
First incidence (days)	501	591	667	704
Logistic regression test	P < 0.001N	P=0.002N	P=0.020N	P < 0.001N

(T) Terminal sacrifice
* Significantly different (P≤0.05) from the control group by the logistic regression test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical incidence for NTP gavage studies with corn oil control groups (mean ± standard deviation): 267/813 (32.8% ± 13.1%); range 14%-58%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the vehicle control group column are the P values associated with the trend test. In the dosed group columns are the P values corresponding to the pairwise comparisons between the vehicle controls and that dosed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in a dosed group is indicated by **N**.
[h] Historical incidence: 140/813 (17.2% ± 5.0%); range 8%-26%
[i] Historical incidence: 364/813 (44.8% ± 14.1%); range 25%-72%
[j] Historical incidence: 111/809 (13.7% ± 8.6%); range 2%-28%
[k] Historical incidence: 145/809 (17.9% ± 9.9%); range 4%-37%

GENETIC TOXICOLOGY

Theophylline in concentrations from 100 to 10,000 μg/plate did not induce mutations in *Salmonella typhimurium* strain TA97, TA98, TA100, or TA1535 when included in the incubation medium with or without induced rat or hamster liver S9 (Table E1; Zeiger *et al.*, 1988). In cytogenetic tests with cultured Chinese hamster ovary cells, theophylline induced sister chromatid exchanges in the absence of S9 activation at concentrations from 100 to 405 μg/mL (Table E2). Cell cycle delay was noted in cultures exposed to concentrations of 300 μg/mL or greater, and incubation time was lengthened accordingly. Theophylline did not induce chromosomal aberrations in cultured Chinese hamster ovary cells, with or without S9 (Table E3).

Theophylline, administered to $B6C3F_1$ mice by intraperitoneal injection for a mouse bone marrow sister chromatid exchange assay, showed a significant, dose-related increase in sister chromatid exchanges at doses of 125 and 250 mg/kg (Table E4; McFee, 1991); however, a repeat trial was not performed, and therefore the response is unconfirmed. Theophylline, administered to $B6C3F_1$ mice by intraperitoneal injection, gave negative results in a mouse bone marrow chromosomal aberrations test that employed both standard (17-hour) and delayed harvest (36-hour) times (Table E5; McFee, 1991). Dose levels were limited by toxicity to 250 mg/kg in the standard harvest time study and 150 mg/kg in the extended harvest time study.

The frequency of micronucleated normochromatic erythrocytes was measured in peripheral blood samples from male and female mice at the termination of the 14-week feed and gavage studies with theophylline (Tables E6 and E7). No significant increases in the frequency of micronucleated erythrocytes were noted in male or female mice.

In conclusion, theophylline showed limited evidence of mutagenicity. Sister chromatid exchanges were observed after treatment of mammalian cells *in vitro* and *in vivo*, but negative results were seen in all other assays.

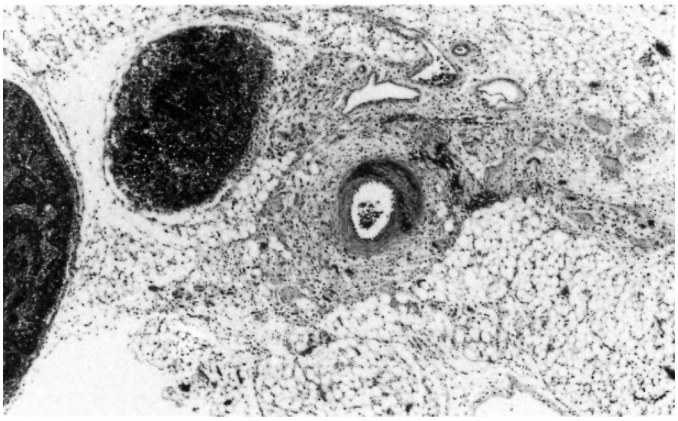


PLATE 1
Hemorrhage and necrosis within the media of a mesenteric artery of a male F344/N rat administered 400 mg theophylline/kg body weight by gavage for 16 days. Note the mixed inflammatory cell infiltrates in the adventitia. H&E; 13.2×

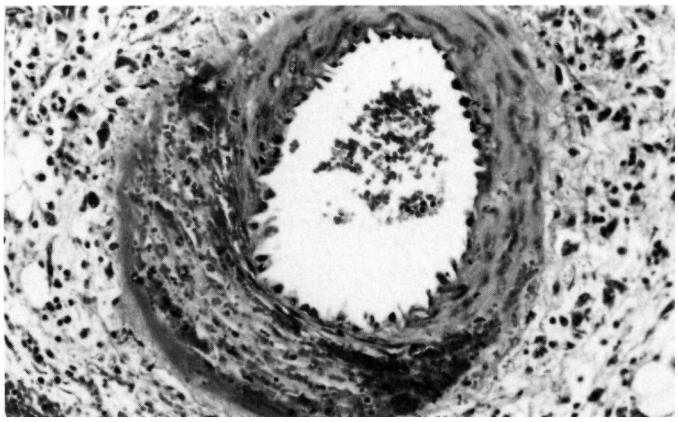


PLATE 2
A higher magnification of Plate 1. H&E; 66×

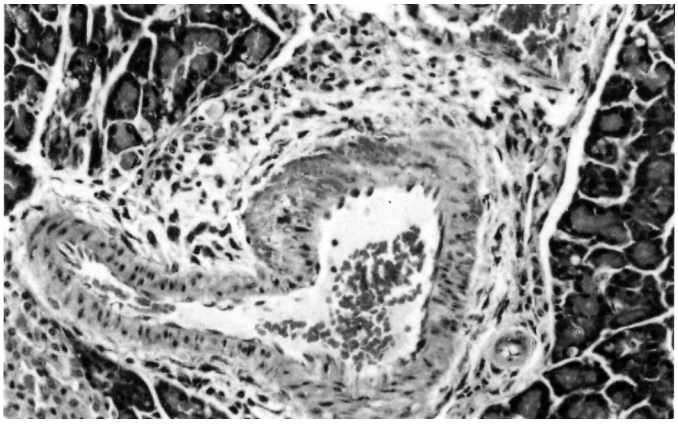


PLATE 3
Hemorrhage and necrosis within the media of a pancreatic artery of a male F344/N rat administered 400 mg theophylline/kg body weight by gavage for 16 days. Note the mixed inflammatory cell infiltrates consisting of polymorphonuclear and mononuclear cells and proliferating fibroblasts in the adventitia. H&E; 66×

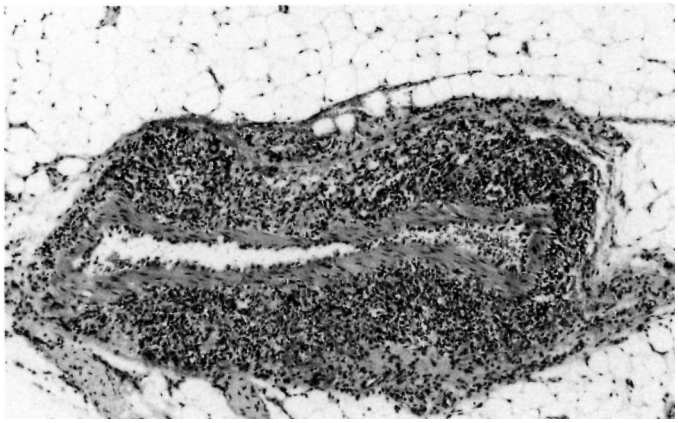


PLATE 4
Periarteritis in the mesenteric artery of a female F344/N rat exposed to 2,000 ppm theophylline in feed for 14 weeks (Collins *et al.*, 1988). Note the periarterial mixed inflammatory cell infiltrates within the adventitia and the outer tunica media. H&E; 120×

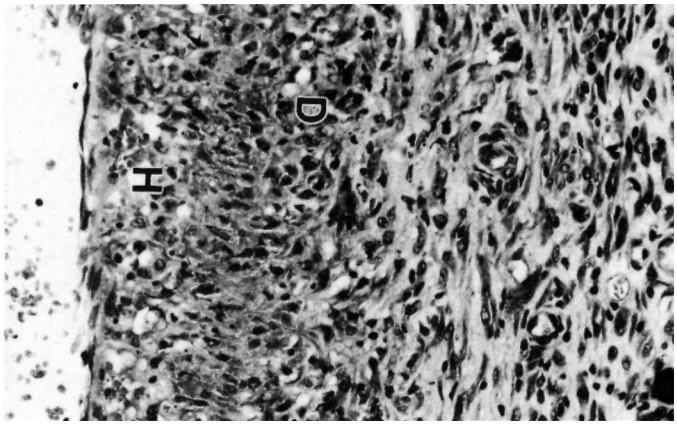


PLATE 5
Periarteritis in the pancreatic artery of a female F344/N rat exposed to 4,000 ppm theophylline in feed for 14 weeks (Collins *et al.*, 1988). Note the mixed inflammatory cell infiltrates within the adventitia and the tunica media. Smooth muscle degeneration (D) and hemorrhage (H) are present in the tunica media. H&E; 384×

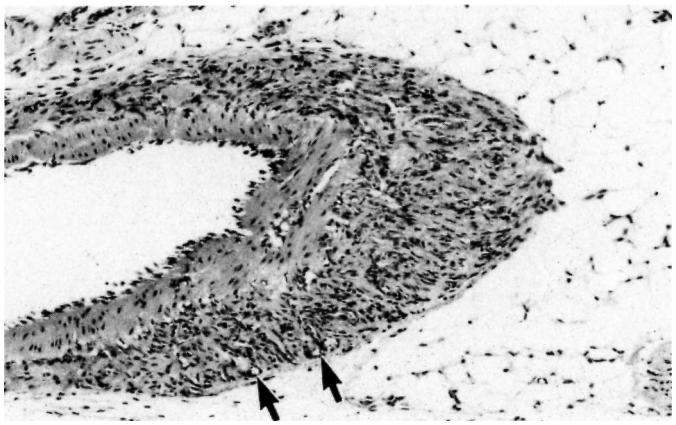


PLATE 6
Periarteritis in the mesenteric artery of a male F344/N rat administered 150 mg theophylline/kg body weight by gavage for 14 weeks (Collins *et al.*, 1988). Note the endothelial lined spaces (arrows) within the fibrous connective tissue in the adventitia in addition to the mixed inflammatory cell infiltrates in the adventitia and the tunica media. H&E; 144×

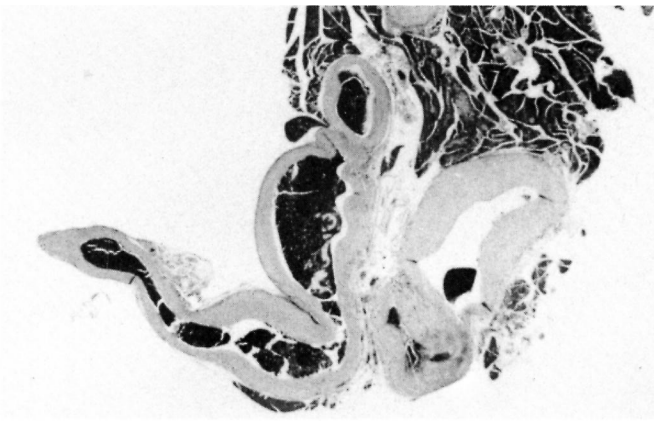


PLATE 7
Subgross appearance of periateritis in the pancreatic arteries of a male F344/N rat administered 75 mg theophylline/kg body weight by gavage for 2 years. Note the thickened and tortuous arterial walls. H&E; 2.5×

PLATE 8
Periarteritis in the pancreatic arteries of a male F344/N rat administered 75 mg theophylline/kg body weight by gavage for 2 years. Note the thickened arterial walls. H&E; 5×

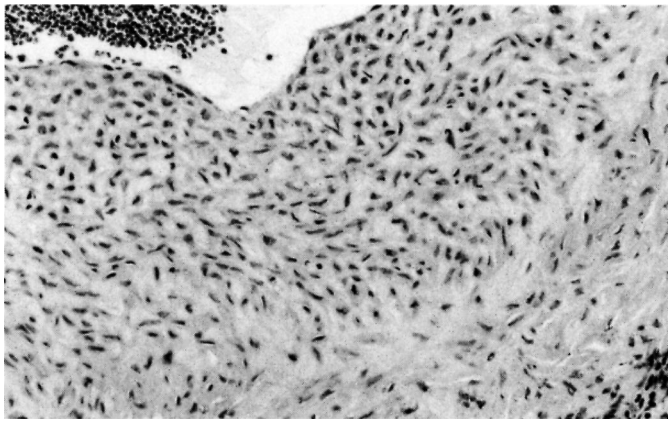


PLATE 9
A higher magnification of Plate 8. Note the proliferation of fibroblasts and infiltration of mixed inflammatory cells in the media and adventitia. H&E; 66×

DISCUSSION AND CONCLUSIONS

Theophylline is widely prescribed in the treatment of obstructive airway diseases but has a low therapeutic index (Minton and Henry, 1996); there is a narrow range between therapeutic doses and doses giving unacceptable toxicity. The rate of administration is a major factor in human fatalities; deaths have resulted from as little as 500 mg aminophylline given in a rapid intravenous injection, and it is recommended that the drug be injected over a 20- to 40-minute period (*Goodman and Gilman's*, 1990). The bolus effect was observed in these studies. In the 16-day studies, all rats and mice receiving 8,000 ppm theophylline (equivalent to 1,000 mg/kg in male rats, 1,100 mg/kg in female rats, 2,000 mg/kg in male mice, and 4,375 mg/kg in female mice) in dosed feed survived, whereas all rats that received 400 mg/kg once daily and nine of 10 rats that received 200 mg/kg twice daily by gavage died. Three of five male mice and all female mice that received once-daily doses of 400 mg/kg died. Rats seemed to be more sensitive to the toxic effects of theophylline than mice, as all mice receiving twice-daily doses of 200 mg/kg survived. Female mice appeared to be more sensitive than male mice; in the 14-week gavage study, all the female mice and three of 10 males that received 300 mg/kg died. In the 2-year studies, survival of male mice that received the highest dose (150 mg/kg) was significantly lower than that of the controls. The highest dose for male and female rats and female mice in the 2-year studies was 75 mg/kg, and there were no significant differences in mortality for these groups compared to controls.

Theophylline generally caused lower body weight gains in rats and mice. The body weight reductions observed could not be accounted for by reduced feed consumption. The body weight effect of theophylline was possibly related to its reported depression of DNA synthesis and antimitotic activity (Timson, 1972). A delay of cell growth has also been reported when phosphodiesterase is inhibited, producing an accumulation of cyclic AMP in the cell (Zajdela and Latarjet, 1978). The diuretic action of theophylline may also contribute to the body weight effect; dehydration was evident in rats based on hematology data in the 16-day feed and gavage studies (increased hematocrit values, hemoglobin concentrations, and erythrocyte counts).

In reproductive and developmental toxicity studies, Weinberger *et al*. (1978) and Friedman *et al*. (1979) reported that theophylline (0.5% in feed), caffeine, and theobromine administered to Holtzman and Osborne-Mendel rats induced bilateral testicular atrophy accompanied by aspermatogenesis or oligospermatogenesis, with the most potent compound being caffeine, then theobromine, and then theophylline. The authors proposed that caffeine and theobromine were more potent than theophylline due to the presence of a methyl group on the N-7 position. Furthermore, in unpublished NTP continuous breeding studies of theophylline, caffeine, and theobromine in Swiss (CD-1®) mice, males treated with theobromine had depressed testicular weight and an increased incidence of abnormal sperm. The studies described in this NTP report showed that testicular atrophy was not observed in F344/N rats or $B6C3F_1$ mice exposed to theophylline in dosed feed at concentrations up to 8,000 ppm for 16 days or 4,000 ppm for 14 weeks, and there were no significant differences in sperm morphology. The testicular effect of theophylline may require higher doses of theophylline and a longer duration of administration than were used in the current NTP studies. It is also possible that the Swiss mouse is more sensitive to the testicular effect of theophylline than is the $B6C3F_1$ mouse.

In the 16-day feed study in rats, uterine hypoplasia occurred in a dose-related manner. In the 16-day gavage study, absolute and relative uterus weights of females receiving 100 or 200 mg/kg once daily were significantly lower than those of the control group; and uterine atrophy was observed in 3 females receiving 200 mg/kg twice daily. However, in the 14-week studies, up to 4,000 ppm theophylline in feed or 150 mg/kg by gavage had no effect on the uterus. It seemed that the uterine effect was transient and required high doses of theophylline. In CD rats and CD-1 mice administered theophylline in drinking water (up to 4,000 ppm for rats and 2,000 ppm for

mice) during gestation days 6 through 15, theophylline induced reductions in gravid uterine weight (George *et al.*, 1986).

In the NTP continuous breeding studies of theophylline, caffeine, and theobromine in Swiss (CD-1®) mice, 3,000 ppm theophylline in feed induced a significant decrease in the number of litters per fertile pair, a dose-related decrease in the number of live pups per litter, and a decreased proportion of pups born alive. Females appeared to be more sensitive to the effects of theophylline than males, as evidenced by results of the cross-breeding studies. Caffeine at concentrations up to 0.05% in drinking water had no effect on fertility, whereas theobromine at concentrations up to 0.5% in feed resulted in significantly decreased fertility and offspring survival (NTP, unpublished report).

The potential carcinogenicity of theophylline was examined using a medium-term liver bioassay based on the induction of glutathione S-transferase placental form-positive foci in F344 rats (Hasegawa *et al.*, 1995). The diethylnitrosamine-initiated and partially hepatectomized rats were given 3,000 ppm theophylline in drinking water. Theophylline had no effect on focus development, suggesting that the chemical was not carcinogenic in rat liver. This finding was in agreement with the current 2-year gavage study of theophylline in F344/N rats.

Dose-related decreases in the incidences of mammary gland fibroadenoma and fibroadenoma or carcinoma (combined) were observed in female rats in the 2-year study. Mammary gland neoplasms in female F344/N rats are known to be correlated with body weight, and application of the Seilkop logistic regression model indicated that the lower body weights in dosed animals could explain the decreased mammary gland neoplasm incidences observed in these groups (Seilkop, 1995; Sheldon *et al.*, 1995; Haseman and Johnson, 1996). That is, the mammary gland neoplasm incidences observed in 25 and 75 mg/kg females are very similar to the neoplasm rates expected in control animals of equivalent size and survival.

Dose-related decreases in the incidences of hepatocellular adenoma and of adenoma or carcinoma (combined) occurred in male and female mice. Theophylline has been shown to depress colony formation and 3H-thymidine incorporation in cultured HT-29 human adenocarcinoma cells (Murnane *et al.*, 1981) and to have an antimitotic effect in cultured human lymphocytes (Timson, 1972). The neoplasm inhibiting effects of theophylline could possibly be related to its interference with normal DNA/nucleotide metabolism.

Although liver neoplasms in $B6C3F_1$ mice are known to be correlated with body weight, application of a logistic regression model (Seilkop, 1995) indicated that lower body weights in the dosed animals and reduced survival (in 150 mg/kg males) could not totally account for the marked reduction in liver neoplasm incidence observed in dosed mice (Table 24). Thus, it is likely that these decreased tumor incidences were chemically related.

Based on retrospective analyses, *Helicobacter hepaticus* was determined to have infected mice in 12 recent NTP 2-year studies (Appendix L). Of the 12 studies, mice (primarily males) from nine studies (including this study of theophylline) had an *H. hepaticus*-associated hepatitis. Qualitatively, the hepatitis and silver-staining organisms within the liver were similar among the nine studies. *H. hepaticus* was identified by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based assay in studies from which adequately preserved (frozen) liver tissue was available. In general, efforts to identify *H. hepaticus* from tissue fixed in formalin for over a week were not successful (Malarkey *et al.*, 1997). However, formalin-fixed liver from one animal from this study of theophylline was positive, with *H. hepaticus* the most likely species. Although this is an uncertain finding, because of the presence of the typical liver lesions and silver-positive helical organisms, mice from this study were presumed to be infected with *H. hepaticus*.

Increases in the incidences of hepatocellular neoplasms in male mice have been shown to be associated with *H. hepaticus* infection when hepatitis is also present (Ward *et al.*, 1994; Fox *et al.*, 1996; Appendix L). Additionally, in NTP studies with *Helicobacter*-associated hepatitis, increased incidences of hemangiosarcoma were seen in the livers of male mice (Table C3). Because of the former association, interpretation of the decreased incidences of hepatocellular neoplasms in the liver of male mice was made more difficult. Incidences of lesions at other sites in this study of theophylline were not considered to have

been significantly impacted by the infection with *H. hepaticus* or its associated hepatitis (Appendix L).

In the present studies, periarteritis was noted in rats administered theophylline for 16 days by gavage, for 14 weeks in feed or by gavage, or for 2 years by gavage. The incidences generally followed a dose-related trend, and the incidences in exposed females in the 14-week feed study and in dosed males in the 2-year gavage study were significantly different from the respective control incidences.

The F344 rat has a low incidence of spontaneous vascular disease, and the vascular system is not a common site for chemically induced lesions in the rat, except following exposure to certain vasoactive compounds including angiotensin, norepinephrine, dopamine agonists, and xanthine compounds (Mitsumori, 1990). The incidence of spontaneous polyarteritis (synonyms: polyarteritis nodosa, periarteritis nodosa, chronic arteritis, necrotizing arteritis, necrotizing vasculitis) varies among different rat strains but is relatively uncommon in the F344 rat (Mitsumori, 1990; Carlton and Engelhardt, 1991), with incidences of 1.8% in males and 0.9% in females. Spontaneous polyarteritis is most commonly seen in the pancreatic, mesenteric, and spermatic arteries (Mitsumori, 1990).

Spontaneous polyarteritis is characterized in the early stages by fibrinoid necrosis of the media and internal elastic lamellae, followed by inflammatory mixed cell infiltrates into the media and adventitia consisting mainly of neutrophils (Mitsumori, 1990; Carlton and Engelhardt, 1991). As lesions progress, medial and adventitial fibrosis become prominent, and the infiltrates shift to a mixture of mononuclear cells such as lymphocytes, plasma cells, and monocytes, with only a few neutrophils and eosinophils present. These cells often encircle small arteries and can obliterate them. Lesions in larger arteries may result in thrombosis, aneurysmal dilatation, or intimal cell proliferation. Macrophages around affected arteries may contain hemosiderin pigment. The cause of spontaneous polyarteritis in rats is unknown, but the lesions resemble the immune-mediated arteritis observed in other species and associated with deposition of antigen-antibody complexes in the arterial walls plus degeneration and necrosis of the vessel wall (Mitsumori, 1990).

Lesions similar to spontaneous polyarteritis were noted in the mesenteric vessels of Sprague-Dawley rats given caffeine in feed for up to 117 weeks (Johansson, 1981); in the small and medium-sized arteries of the pancreas, lymph node, kidney, and stomach of rats given LY-195115 (an experimental inotropic agent) in feed for 3 months (Sandusky and Means, 1987); in the large mesenteric arteries of rats administered fenoldopam mesylate (a postsynaptic DA_1 dopaminergic vasodilator) intravenously for 24 hours (Kerns *et al.*, 1989); in the small and large arteries of the mesentery, cerebrum, heart, and kidney of rats administered dopamine intravenously for 24 hours (Kerns *et al.*, 1989); in rats administered a variety of structurally unrelated vasodilators (Mitsumori, 1990; Carlton and Engelhardt, 1991; Kerns *et al.*, 1991); and in male Wistar rats administered one of four phosphodiesterase III (PDE III) inhibitors in a single subcutaneous dose (Joseph *et al.*, 1996). The lesions induced by PDE III inhibitors administered to rats were similar to lesions induced by PDE III inhibitors administered to dogs, except for the site of predilection (Boor *et al.*, 1995) In rats, the lesions occurred in the mesentery rather than coronary vasculature.

It has been proposed that the arteriopathy induced by structurally unrelated vasodilators is the result of disturbances in critical wall tension due to relaxation of the medial smooth muscle (Carlton and Engelhardt, 1991). The supposition is that smooth muscle cell necrosis results because the prolonged reduction in the blood pressure coupled with alteration in intramural tension may interfere with the diffusion of essential nutrients into the wall of affected arteries, increasing susceptibility to injury (Bugelski *et al.*, 1989). However, Bugelski *et al*. (1989) reported infusing rats in other experiments with hydralazine or sodium nitroprusside, each of which produced a profound reduction in blood pressure, but found no evidence of arterial lesions.

It has also been proposed that arterial necrosis develops due to alternating vasodilation and vasoconstriction of the arterial smooth muscles (Yuhas *et al.*, 1985; Bugelski *et al.*, 1989). Using electron microscopy to examine lesions in male Sprague-Dawley CD rats administered fenoldopam mesylate by infusion for 24 hours, Bugelski *et al*. (1989) observed

pseudovacuoles or blebbing of the plasmalemma and extrusion of cytoplasm between adjacent smooth muscle cells. Occasionally, extruded nuclei were observed.

Continuous infusion of fenoldopam mesylate in rats produced medial necrosis and hemorrhage in small and medium-sized renal (arcuate, hilar, and interlobar) arteries and mesenteric arteries (such as the interlobular arteries of the pancreas and subserosal arteries of the stomach, duodenum, jejunum, ileum, and colon) (Yuhas *et al.*, 1985; Bugelski *et al.*, 1989). Kerns *et al*. (1989) found that dopamine, though generally considered a vasoconstrictor, acts as a vasodilator in the mesenteric arteries. The authors infused male Charles River CD rats with fenoldopam mesylate or dopamine, a dopaminergic and α- and β-adrenergic receptor agonist. Fenoldopam mesylate caused lesions of the large mesenteric arteries characterized by necrosis of medial smooth muscle cells and hemorrhage. Dopamine caused hemorrhagic lesions of the large mesenteric arteries and fibrinoid necrosis of the small arteries in the mesentery, cerebrum, heart, and kidney. Coadministration of a DA_1 antagonist with fenoldopam or dopamine blocked the development of periarteritis of the large arteries. Coadministration of an α-adrenergic antagonist with fenoldopam or dopamine blocked the dopamine-induced development of fibrinoid lesions in the small arteries but increased the severity of lesions in the large arteries induced by fenoldopam mesylate or dopamine. Coadministration of a DA_1 antagonist and an α-adrenergic antagonist with dopamine blocked the development of the lesions characteristic of dopamine exposure.

Joseph *et al*. (1996) subcutaneously administered four structurally dissimilar PDE III inhibitors to male Wistar rats and induced lesions similar to those induced by fenoldopam mesylate. The lesions were characterized by medial necrosis and hemorrhage, occurred with a dose-related intensity, and correlated well with the degree of hypotension induced by each of the PDE III inhibitors. The first changes were observed in the muscular mesenteric arteries with an external diameter of 100 to 800 µm and appeared within 6 hours of dosing. The endothelial cells appeared raised and the interendothelial projections were more pronounced. Discrete foci of erythrocytes appeared within the media immediately adjacent to the internal elastic lamellae, with necrosis of one or two smooth muscle cells. The medial cells located away from the immediate vicinity of the erythrocytes had a normal range of structures. At 16 hours after dosing, the medial hemorrhage and necrosis was more extensive and segmental. Loss of endothelial cells was evident at this stage, and leukocytes and activated platelets were found adhering to the exposed basement membrane and within the subintima. Joseph *et al*. (1996) also postulated that the arterial damage was a consequence of profound vasodilatation resulting in abnormal endothelial permeability and increased wall tension resulting in progressive medial necrosis and hemorrhage. Both dopaminergic DA_1 agonists and PDE III inhibitors induce the same biochemical event related to their vasodilative effect, namely, increased cAMP in the arterial smooth muscles (Joseph *et al.*, 1996).

In summary, the small and medium-sized mesenteric and pancreatic arteries in rats are particularly sensitive to the excessive vasodilator-pharmacologic activity of theophylline, as well as caffeine (Johansson, 1981) and other vasodilator drugs (Kerns *et al.*, 1991; Boor *et al.*, 1995). In the present studies, the incidence of the lesions was dose related, which further supports a pharmacologic etiology rather than hypersensitivity. The reason for the particular predisposition of effects in the medium-sized arteries in the mesenteric and pancreatic vascular beds in the rat is probably related to the morphology of these vessels and to the localization of particular receptors to the drug in this location (Nordborg *et al.*, 1985; Kerns *et al.*, 1989; Greaves, 1990).

The severity of nephropathy was greater in male rats exposed to 4,000 ppm for 14 weeks than in the controls or other exposed groups. Although no renal vascular morphological changes were recorded in the present studies, it has been mentioned that vasodilator drugs like fenoldopam mesylate induce pathological changes in the renal arteries (Yuhas *et al.*, 1985; Kerns *et al.*, 1989). An intentional human overdose (fifty 300 mg tablets) of theophylline induced acute renal failure for which three pathophysiological mechanisms were proposed: severe renal vasoconstriction, myoglobinuria, or adenosine antagonism (ter Maaten and Hoorntje, 1993).

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of theophylline in male or female F344/N rats administered 7.5, 25, or 75 mg/kg. There was *no evidence of carcinogenic activity* of theophylline in male $B6C3F_1$ mice administered 15, 50, or 150 mg/kg or female $B6C3F_1$ mice administered 7.5, 25, or 75 mg/kg.

Gavage administration of theophylline caused chronic inflammation of the mesenteric arteries in dosed male rats.

Decreased incidences of mammary neoplasms in female rats were likely associated with lower body weights. There were dose-related decreases in the incidences of hepatocellular adenoma and hepatocellular carcinoma in male and female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Review Subcommittee comments and the public discussion on this Technical report appears on page 12.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR GAVAGE STUDY OF THEOPHYLLINE

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1	1	1	
Moribund	21	11	18	13
Natural deaths	5	5	2	13
Survivors				
Terminal sacrifice	23	33	29	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine small, jejunum	(49)	(48)	(50)	(49)
Polyp adenomatous				1 (2%)
Intestine small, ileum	(49)	(48)	(50)	(49)
Liver	(50)	(50)	(50)	(50)
Fibrous histiocytoma		1 (2%)		
Hepatocellular adenoma	1 (2%)	2 (4%)		1 (2%)
Hepatocellular adenoma, multiple	1 (2%)			
Histiocytic sarcoma	2 (4%)			
Osteosarcoma, metastatic, bone		1 (2%)		
Mesentery	(16)	(16)	(17)	(27)
Histiocytic sarcoma	1 (6%)			
Oral mucosa	(1)		(1)	
Squamous cell papilloma	1 (100%)			
Pancreas	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Acinus, adenoma	8 (16%)	6 (12%)	4 (8%)	4 (8%)
Acinus, adenoma, multiple	2 (4%)	1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Schwannoma malignant, metastatic, skin		1 (2%)		
Tongue	(1)		(1)	(1)
Squamous cell papilloma	1 (100%)		1 (100%)	
Cardiovascular System				
None				
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma	1 (2%)			
Adrenal medulla	(50)	(49)	(50)	(50)
Fibrous histiocytoma		1 (2%)		
Pheochromocytoma malignant		2 (4%)		
Pheochromocytoma malignant, multiple	1 (2%)			
Pheochromocytoma complex		1 (2%)		
Pheochromocytoma benign	5 (10%)	5 (10%)	6 (12%)	9 (18%)
Bilateral, pheochromocytoma benign	2 (4%)		1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Carcinoma	2 (4%)	1 (2%)		
Carcinoma, multiple	1 (2%)			

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(49)	(50)	(48)
Pars distalis, adenoma	19 (38%)	18 (37%)	16 (32%)	10 (21%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	5 (10%)	1 (2%)	3 (6%)	1 (2%)
C-cell, carcinoma	1 (2%)	2 (4%)		
Follicular cell, adenoma				1 (2%)
Follicular cell, carcinoma			2 (4%)	1 (2%)
General Body System				
Peritoneum	(2)	(2)	(3)	(3)
Fibrosarcoma, metastatic, spleen	1 (50%)			
Tissue NOS		(2)	(1)	
Mediastinum, hemangioma		1 (50%)		
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(49)	(50)	(50)
Adenoma		2 (4%)	4 (8%)	3 (6%)
Carcinoma	3 (6%)	2 (4%)		3 (6%)
Bilateral, carcinoma				1 (2%)
Prostate	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	42 (84%)	38 (76%)	41 (82%)	43 (86%)
Interstitial cell, adenoma	4 (8%)	4 (8%)	1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(13)	(11)	(12)	(9)
Inguinal, fibrous histiocytoma		1 (9%)		
Mediastinal, fibrous histiocytoma		1 (9%)		
Mediastinal, histiocytic sarcoma	1 (8%)			
Lymph node, mandibular	(48)	(49)	(50)	(50)
Osteosarcoma, metastatic, bone				1 (2%)
Lymph node, mesenteric	(50)	(49)	(50)	(49)
Fibrous histiocytoma		1 (2%)		
Histiocytic sarcoma	1 (2%)			
Spleen	(49)	(50)	(50)	(49)
Fibroma		2 (4%)	1 (2%)	
Fibrosarcoma	1 (2%)			
Fibrous histiocytoma		1 (2%)		
Hemangiosarcoma				1 (2%)
Thymus	(49)	(47)	(47)	(50)
Thymoma benign			1 (2%)	
Thymoma malignant			1 (2%)	

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Integumentary System				
Mammary gland	(48)	(49)	(47)	(48)
Carcinoma	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Fibroadenoma	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Basal cell adenoma		1 (2%)		
Keratoacanthoma	2 (4%)	1 (2%)		
Squamous cell papilloma			1 (2%)	
Trichoepithelioma		1 (2%)		
Subcutaneous tissue, fibroma	5 (10%)	1 (2%)	6 (12%)	4 (8%)
Subcutaneous tissue, fibroma, multiple	1 (2%)			1 (2%)
Subcutaneous tissue, fibrosarcoma		1 (2%)		
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, histiocytic sarcoma	1 (2%)			
Subcutaneous tissue, lipoma				1 (2%)
Subcutaneous tissue, melanoma benign		1 (2%)		
Subcutaneous tissue, melanoma malignant		2 (4%)		
Subcutaneous tissue, schwannoma malignant		1 (2%)	1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma		1 (2%)		1 (2%)
Skeletal muscle	(4)	(1)	(1)	
Fibrosarcoma, metastatic, spleen	1 (25%)			
Histiocytic sarcoma	1 (25%)			
Sarcoma			1 (100%)	
Nervous System				
Brain	(50)	(49)	(50)	(49)
Astrocytoma malignant		1 (2%)		
Glioma malignant		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)		1 (2%)	2 (4%)
Alveolar/bronchiolar carcinoma	1 (2%)			
Fibrous histiocytoma		1 (2%)		
Histiocytic sarcoma	2 (4%)			
Osteosarcoma, metastatic, bone		1 (2%)		
Osteosarcoma, metastatic, uncertain primary site	1 (2%)			
Special Senses System				
Zymbal's gland			(2)	
Carcinoma			2 (100%)	

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Urinary System				
Kidney	(49)	(49)	(50)	(49)
Fibrous histiocytoma		1 (2%)		
Histiocytic sarcoma	1 (2%)			
Artery, osteosarcoma, metastatic, bone		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	2 (4%)			
Leukemia mononuclear	15 (30%)	5 (10%)	6 (12%)	6 (12%)
Mesothelioma malignant	1 (2%)	2 (4%)	4 (8%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	49	48	47
Total primary neoplasms	134	118	106	100
Total animals with benign neoplasms	49	49	48	46
Total benign neoplasms	104	87	89	84
Total animals with malignant neoplasms	23	18	14	14
Total malignant neoplasms	30	31	17	16
Total animals with metastatic neoplasms	2	2		1
Total metastatic neoplasms	5	4		1
Total animals with malignant neoplasms of uncertain primary site	1			

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: Vehicle Control

Number of Days on Study	3 8 8	5 2 9	5 3 3	5 4 5	5 4 6	5 7 2	5 9 8	6 0 7	6 1 4	6 1 6	6 1 7	6 1 7	6 3 8	6 3 8	6 4 2	6 4 9	6 4 9	6 5 8	6 7 0	6 7 0	6 7 1	7 0 1	7 0 0	7 1 9	7 2 6
Carcass ID Number	0 3 7	0 0 1	0 4 5	0 0 5	0 1 4	0 3 1	0 2 9	0 1 2	0 2 7	0 1 8	0 2 0	0 3 9	0 0 6	0 4 0	0 4 7	0 1 0	0 3 5	0 4 2	0 1 1	0 1 6	0 3 3	0 4 6	0 2 4	0 1 5	0 3 8
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma					X																				
Hepatocellular adenoma, multiple																									
Histiocytic sarcoma																								X	
Mesentery				+	+	+	+	+				+			+	+						+			
Histiocytic sarcoma																									
Oral mucosa											+														
Squamous cell papilloma											X														
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Acinus, adenoma																				X					
Acinus, adenoma, multiple																X									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																					+				
Squamous cell papilloma																					X				
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																					X				
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant, multiple																									
Pheochromocytoma benign							X									X									
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Carcinoma, multiple																									X
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X	X			X	X	X				X	X							X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma					X		X													X				
C-cell, carcinoma																									

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	7 2 7	7 2 8	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 3 2	0 1 3	0 0 2	0 0 3	0 0 4	0 0 7	0 0 8	0 0 9	0 1 7	0 1 9	0 2 1	0 2 2	0 2 3	0 2 5	0 2 6	0 2 8	0 3 0	0 3 4	0 3 6	0 4 1	0 4 3	0 4 4	0 4 8	0 4 9	0 5 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																										1
Hepatocellular adenoma, multiple																						X				1
Histiocytic sarcoma																			X							2
Mesentery			+		+					+		+			+	+			+							16
Histiocytic sarcoma																			X							1
Oral mucosa																										1
Squamous cell papilloma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																			X							1
Acinus, adenoma			X	X	X				X	X					X										X	8
Acinus, adenoma, multiple																	X									2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Squamous cell papilloma																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant, multiple			X																							1
Pheochromocytoma benign									X				X	X												5
Bilateral, pheochromocytoma benign						X																X				2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma		X														X										2
Carcinoma, multiple																										1
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X	X		X					X			X		X			X					X	X	X	19
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma			X																X							5
C-cell, carcinoma																X										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	3 8 8	5 2 9	5 3 3	5 4 5	5 4 6	5 7 2	5 9 8	6 0 7	6 1 4	6 1 6	6 1 7	6 1 7	6 3 8	6 3 8	6 4 2	6 4 9	6 4 9	6 5 8	6 7 0	6 7 0	6 7 1	6 7 1	7 0 0	7 1 9	7 2 6
Carcass ID Number	0 3 7	0 0 1	0 4 5	0 0 5	0 1 4	0 3 1	0 2 9	0 1 2	0 2 7	0 1 8	0 2 0	0 3 9	0 0 6	0 4 0	0 4 7	1 0	3 5	4 2	1 1	1 6	3 3	4 6	2 4	1 5	3 8
General Body System																									
Peritoneum						+								+											
Fibrosarcoma, metastatic, spleen														X											
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, peritoneum						X																			
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma						X																X	X		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X	X	X	X	X		X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	
Interstitial cell, adenoma																		X							
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+	+	+			+		+				+		+					+				
Mediastinal, histiocytic sarcoma																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	I	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								X	
Spleen	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma														X											
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Carcinoma																									
Fibroadenoma																			X	X					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																							X		
Subcutaneous tissue, fibroma									X										X	X					
Subcutaneous tissue, fibroma, multiple																									
Subcutaneous tissue, histiocytic sarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle						+		+						+											
Fibrosarcoma, metastatic, spleen														X											
Histiocytic sarcoma																									
Mesothelioma malignant, metastatic, peritoneum						X																			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	7 2 7	7 2 8	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 3 2	0 1 3	0 0 2	0 0 3	0 0 4	0 0 7	0 0 8	0 0 9	0 1 7	0 1 9	0 2 1	0 2 2	0 2 3	0 2 5	0 2 6	0 2 8	0 3 0	0 3 4	0 3 6	0 4 1	0 4 3	0 4 4	0 4 8	0 4 9	0 5 0	Total Tissues/ Tumors
General Body System																										
Peritoneum																										2
Fibrosarcoma, metastatic, spleen																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, peritoneum																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																			X							1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma		X	X	X		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	42
Interstitial cell, adenoma	X				X				X																	4
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+				+											+			+				+			13
Mediastinal, histiocytic sarcoma																			X							1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrosarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	48
Carcinoma		X																								1
Fibroadenoma		X																								3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma			X																							2
Subcutaneous tissue, fibroma		X										X														5
Subcutaneous tissue, fibroma, multiple	X																									1
Subcutaneous tissue, histiocytic sarcoma																			X							1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																			+							4
Fibrosarcoma, metastatic, spleen																										1
Histiocytic sarcoma																			X							1
Mesothelioma malignant, metastatic, peritoneum																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	3 8 8	5 2 9	5 3 3	5 4 5	5 4 6	5 7 2	5 9 8	6 0 7	6 1 4	6 1 6	6 1 7	6 1 7	6 3 8	6 3 8	6 4 2	6 4 9	6 4 9	6 5 8	6 7 0	6 7 0	6 7 1	6 7 1	7 0 0	7 1 9	7 2 6
Carcass ID Number	0 3 7	0 0 1	0 4 5	0 0 5	0 1 4	0 3 1	0 2 9	0 1 2	0 2 7	0 1 8	0 2 0	0 3 9	0 0 6	0 4 0	0 4 7	0 1 0	0 3 5	0 4 2	0 1 1	0 1 6	0 3 3	0 4 6	0 2 4	0 1 5	0 3 8
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X																
Alveolar/bronchiolar carcinoma																									
Histiocytic sarcoma																								X	
Osteosarcoma, metastatic, uncertain primary site						X																			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye				+																					
Harderian gland																								+	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								X	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								X	
Leukemia mononuclear		X		X	X			X		X			X	X		X		X	X		X				X
Mesothelioma malignant						X																			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	7 2 7	7 2 8	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 3 2	0 1 3	0 0 2	0 0 3	0 0 4	0 0 7	0 0 8	0 0 9	0 1 7	0 1 9	0 2 1	0 2 2	0 2 3	0 2 5	0 2 6	0 2 8	0 3 0	0 3 4	0 3 6	0 4 1	0 4 3	0 4 4	0 4 8	0 4 9	0 5 0	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																					X					2
Alveolar/bronchiolar carcinoma				X																						1
Histiocytic sarcoma																			X							2
Osteosarcoma, metastatic, uncertain primary site																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+				+	+	+					+				+								I	8
Harderian gland																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																			X							2
Leukemia mononuclear				X										X								X				15
Mesothelioma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg

Number of Days on Study	2 3 4	3 7 2	4 9 7	5 0 0	5 4 7	5 5 8	5 6 6	6 1 6	6 1 6	6 2 4	6 4 4	6 6 6	6 6 7	7 0 6	7 1 3	7 1 9	7 2 3	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 7 6	0 9 7	0 7 3	0 6 1	0 7 1	0 6 5	0 6 0	0 9 4	1 0 0	0 9 5	0 5 9	0 9 1	0 6 4	0 9 8	0 5 7	0 5 8	0 5 2	0 5 1	0 5 3	0 5 4	0 6 3	0 7 0	0 7 2	0 7 4	0 8 8
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma											X														
Hepatocellular adenoma																									
Osteosarcoma, metastatic, bone												X													
Mesentery			+				+			+				+	+							+			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																X							X		
Acinus, adenoma, multiple																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, skin						X																			
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma											X														
Pheochromocytoma malignant				X																					
Pheochromocytoma complex																									
Pheochromocytoma benign																	X						X		
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																			X						
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X		X		X		X			X	X	X	X		X					X			X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
C-cell, carcinoma														X											
General Body System																									
Peritoneum										+					+										
Tissue NOS									+																
Mediastinum, hemangioma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	729	729	730	730	730	730	730	730	730	730	730	730	730	733	733	733	733	733	734	734	734	734	734	734	
Carcass ID Number	0090	0092	0055	0056	0062	0075	0077	0078	0079	0089	0093	0096	0099	0066	0067	0068	0069	0080	0081	0082	0083	0084	0085	0086	0087	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma																										1
Hepatocellular adenoma																		X		X						2
Osteosarcoma, metastatic, bone																										1
Mesentery						+		+	+	+		+		+	+	+		+						+		16
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma	X														X		X		X							6
Acinus, adenoma, multiple																						X				1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant, metastatic, skin																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	49
Fibrous histiocytoma																										1
Pheochromocytoma malignant								X																		2
Pheochromocytoma complex																			X							1
Pheochromocytoma benign								X							X	X										5
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma						X	X			X		X			X				X						X	18
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																	X									1
C-cell, carcinoma																									X	2
General Body System																										
Peritoneum																										2
Tissue NOS											+															2
Mediastinum, hemangioma											X															1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	2 3 4	3 7 2	4 9 7	5 0 0	5 4 7	5 5 8	5 6 6	6 1 6	6 1 6	6 2 4	6 4 4	6 6 6	6 6 7	7 0 6	7 1 3	7 1 9	7 2 3	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 0 7 6	0 0 9 7	0 0 7 3	0 0 6 1	0 0 7 1	0 0 6 5	0 0 6 0	0 1 9 4	0 1 0 0	0 1 0 9	0 0 5 9	0 0 9 1	0 0 6 4	0 0 9 8	0 0 5 7	0 0 5 8	0 0 5 2	0 0 5 1	0 0 5 3	0 0 5 4	0 0 6 3	0 0 7 0	0 0 7 2	0 0 7 4	0 0 8 8
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Adenoma												X													
Carcinoma																									X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X				X		X		X		X		X	X		X	X	X	X	X	X	X	X	X
Interstitial cell, adenoma				X							X														
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+				+	+	+		+	+					+	+				+
Inguinal, fibrous histiocytoma											X														
Mediastinal, fibrous histiocytoma											X														
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma											X														
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
Fibrous histiocytoma											X														
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																		X							
Keratoacanthoma																									
Trichoepithelioma																									
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibrosarcoma																				X					
Subcutaneous tissue, fibrous histiocytoma											X														
Subcutaneous tissue, melanoma benign															X										
Subcutaneous tissue, melanoma malignant																									
Subcutaneous tissue, schwannoma malignant						X																			
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma												X													
Skeletal muscle														+											
Nervous System																									
Brain	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant		X																							
Glioma malignant																					X				
Peripheral nerve												+													
Spinal cord									+																

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	729	729	730	730	730	730	730	730	730	730	730	730	730	730	733	733	733	733	733	733	734	734	734	734	734	734	
Carcass ID Number	09 0	09 2	05 5	05 6	06 2	07 5	07 7	07 8	07 9	08 9	09 3	09 6	09 9	09 9	06 6	06 7	06 8	06 9	08 0	08 1	08 2	08 3	08 4	08 5	08 6	08 7	Total Tissues/ Tumors
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma						X																					2
Carcinoma									X																		2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X	X	X	38
Interstitial cell, adenoma																				X			X				4
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node					+					+																	11
Inguinal, fibrous histiocytoma																											1
Mediastinal, fibrous histiocytoma																											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrous histiocytoma																											1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroma						X											X										2
Fibrous histiocytoma																											1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibroadenoma	X																				X						2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																											1
Keratoacanthoma						X																					1
Trichoepithelioma			X																								1
Subcutaneous tissue, fibroma																								X			1
Subcutaneous tissue, fibrosarcoma																											1
Subcutaneous tissue, fibrous histiocytoma																											1
Subcutaneous tissue, melanoma benign																											1
Subcutaneous tissue, melanoma malignant																								X		X	2
Subcutaneous tissue, schwannoma malignant																											1
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																											1
Skeletal muscle																											1
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Astrocytoma malignant																											1
Glioma malignant																											1
Peripheral nerve																											1
Spinal cord																											1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	2 3 4	3 7 2	4 9 7	5 0 0	5 4 7	5 5 8	5 6 6	6 1 6	6 1 6	6 2 4	6 4 4	6 6 6	6 6 7	7 0 6	7 1 3	7 1 9	7 2 3	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 7 6	0 9 7	0 7 3	0 6 1	0 7 1	0 6 5	0 6 0	0 9 4	1 0 0	0 9 5	0 5 9	0 9 1	0 6 4	0 9 8	0 5 7	0 5 8	0 5 2	0 5 1	0 5 3	0 5 4	0 6 3	0 7 0	0 7 2	0 7 4	0 8 8
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma											X														
Osteosarcoma, metastatic, bone												X													
Nose	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									+
Lacrimal gland		+																							
Urinary System																									
Kidney	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma											X														
Artery, osteosarcoma, metastatic, bone												X													
Ureter																+									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear														X	X					X					
Mesothelioma malignant										X					X										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	729	729	730	730	730	730	730	730	730	730	730	730	730	730	733	733	733	733	733	733	734	734	734	734	734	734	
Carcass ID Number	0909	0912	0955	0956	0962	0975	0977	0978	0979	0989	0993	0996	0999	0966	0667	0668	0669	0680	0681	0682	0683	0684	0685	0686	0687		Total Tissues/ Tumors
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Fibrous histiocytoma																											1
Osteosarcoma, metastatic, bone																											1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Special Senses System																											
Eye	+	+	+						+							+							+				7
Lacrimal gland																											1
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Fibrous histiocytoma																											1
Artery, osteosarcoma, metastatic, bone																											1
Ureter																											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Leukemia mononuclear		X							X																		5
Mesothelioma malignant																											2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 25 mg/kg

Number of Days on Study	2 3 3	4 2 2	4 2 5	4 9 8	4 9 8	5 2 0	5 2 6	5 5 8	5 5 8	5 6 0	5 7 2	5 9 0	6 1 6	6 1 7	6 2 2	6 3 9	6 4 7	6 6 6	6 7 0	6 8 0	7 0 6	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 4 1	1 1 0	1 0 8	1 1 4	1 1 8	1 3 2	1 3 1	1 3 8	1 4 0	1 4 5	1 0 3	1 3 4	1 1 9	1 3 6	1 1 5	1 2 3	1 3 9	1 1 1	1 0 2	1 2 7	1 3 5	1 3 0 1	1 0 4	1 1 2	1 2 0
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery		+				+			+	+			+	+	+			+					+		
Oral mucosa															+										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Squamous cell papilloma																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																								X	
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X		X				X		X			X	X				X				X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma							X					X													
Follicular cell, carcinoma																									
General Body System																									
Peritoneum					+																				
Tissue NOS						+																			
Genital System																									
Coagulating gland					+																				
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X														X							
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma			X		X		X	X	X		X		X	X	X		X	X	X	X	X	X	X	X	X
Interstitial cell, adenoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 25 mg/kg (continued)

Number of Days on Study	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	733	733	733	734	734	734	
Carcass ID Number	12 1	12 8	13 7	14 2	14 8	10 5	10 6	10 7	11 3	11 6	11 7	12 2	12 4	12 5	12 9	13 0	14 3	14 4	14 6	10 9	12 6	14 7	13 3	14 9	15 0	Total Tissues/ Tumors
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery			+				+	+			+				+					+	+	+						17
Oral mucosa																												1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma								X			X			X							X							4
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue								+																				1
Squamous cell papilloma								X																				1
Cardiovascular System																												
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																												
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X																				1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign						X		X		X				X										X				6
Bilateral, pheochromocytoma benign																						X						1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma			X	X					X				X				X					X	X		X			16
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma											X																	3
Follicular cell, carcinoma					X									X														2
General Body System																												
Peritoneum														+				+										3
Tissue NOS																												1
Genital System																												
Coagulating gland																												1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X													X												4
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	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	41
Interstitial cell, adenoma																				X								1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 25 mg/kg (continued)

Number of Days on Study	2 3 3	4 2 2	4 2 5	4 9 8	4 9 8	5 2 0	5 2 6	5 5 8	5 5 8	5 6 0	5 7 2	5 9 0	6 1 6	6 1 7	6 2 2	6 3 9	6 4 7	6 6 6	6 7 0	6 8 0	7 0 6	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 4 1	1 1 0	1 0 8	1 1 4	1 1 8	1 3 2	1 3 1	1 3 8	1 4 0	1 4 5	1 0 3	1 3 4	1 1 9	1 3 6	1 1 5	1 2 3	1 3 9	1 1 1	1 0 2	1 2 7	1 3 5	1 0 1	1 0 4	1 1 2	1 2 0
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+						+		+		+			+		+	+	+			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign																X									
Thymoma malignant																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	M
Fibroadenoma																						X			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Subcutaneous tissue, fibroma								X										X	X						
Subcutaneous tissue, schwannoma malignant																				X					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																					+				
Sarcoma																					X				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve												+					+			+	+				
Spinal cord												+					+			+	+				
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																					+			+	
Zymbal's gland									+					+											
Carcinoma									X					X											
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra												+													
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear											X		X		X		X		X	X					
Mesothelioma malignant					X						X														

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 25 mg/kg (continued)

Number of Days on Study	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	733	733	733	734	744	734	
Carcass ID Number	12 1	12 8	13 7	14 2	14 8	10 5	10 6	10 7	11 3	11 6	11 7	12 2	12 4	12 5	12 9	13 0	14 3	14 4	14 6	10 9	12 6	14 7	13 3	14 9	15 0			Total Tissues/ Tumors
Hematopoietic System																												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Lymph node			+		+					+												+						12
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Fibroma														X														1
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+			47
Thymoma benign																												1
Thymoma malignant		X																										1
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			47
Fibroadenoma																												1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Squamous cell papilloma			X																									1
Subcutaneous tissue, fibroma								X									X	X										6
Subcutaneous tissue, schwannoma malignant																												1
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Skeletal muscle																												1
Sarcoma																												1
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Peripheral nerve																												4
Spinal cord																												4
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Alveolar/bronchiolar adenoma																	X											1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Special Senses System																												
Eye	+	+			+	+	+			+				+	+	+		+		+		+	+					15
Zymbal's gland																												2
Carcinoma																												2
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Urethra																												1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Leukemia mononuclear																												6
Mesothelioma malignant														X				X										4

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 75 mg/kg

Number of Days on Study	2 1 1	2 4 1	3 0 6	3 8 7	3 8 7	4 4 1	4 5 6	4 5 7	4 5 7	4 5 7	4 5 7	4 5 7	4 6 2	4 9 7	4 9 8	5 5 2	5 6 6	5 7 2	5 7 6	5 9 5	6 4 2	6 4 3	6 4 9	6 7 1	7 0 6
Carcass ID Number	1 1 6 1	1 6 0	1 6 9	1 7 5	1 8 3	1 7 6	1 9 8	1 7 7	1 9 2	1 9 3	1 9 5	2 0 0	1 7 8	1 7 1	1 5 3	1 8 8	1 6 2	1 7 2	1 7 3	1 6 5	1 8 6	1 9 7	1 9 6	1 5 6	1 7 9
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																				X					
Mesentery						+		+	+					+				+	+	+		+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																					X				
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue							+																		
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign											X														X
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pars distalis, adenoma														X											
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																					X				
Follicular cell, adenoma																									
Follicular cell, carcinoma																									
General Body System																									
Peritoneum																+									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma															X				X						
Bilateral, carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma						X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X
Interstitial cell, adenoma					X																				

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 75 mg/kg (continued)

Number of Days on Study	706	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	733	733	733	733	734	734	734	
Carcass ID Number	189	151	152	159	167	170	174	182	184	185	190	194	154	155	157	180	181	191	158	168	187	199	163	164	166	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp adenomatous								X																		1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																										1
Mesentery	+	+	+	+		+		+	+		+			+	+			+	+	+		+	+	+		27
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma			X		X												X									4
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign	X	X				X				X	X		X	X												9
Bilateral, pheochromocytoma benign																									X	1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma			X			X			X						X			X		X		X	X		X	10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																										1
Follicular cell, adenoma																			X							1
Follicular cell, carcinoma																								X		1
General Body System																										
Peritoneum						+							+													3
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma													X								X			X		3
Carcinoma									X																	3
Bilateral, carcinoma										X																1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	43
Interstitial cell, adenoma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 75 mg/kg (continued)

Number of Days on Study	2 1 1	2 4 1	3 0 6	3 8 7	3 8 7	4 4 1	4 5 6	4 5 7	4 5 7	4 5 7	4 5 7	4 5 7	4 6 2	4 9 7	4 9 8	4 5 2	5 6 6	5 7 2	5 7 6	5 9 5	6 4 2	6 4 3	6 4 9	6 7 1	7 0 6
Carcass ID Number	1 6 1	1 6 0	1 6 9	1 7 5	1 8 3	1 7 6	1 9 8	1 7 7	1 9 2	1 9 3	1 9 5	2 0 0	1 7 8	1 7 1	1 5 3	1 8 8	1 6 2	1 7 2	1 7 3	1 6 5	1 8 6	1 9 7	1 9 6	1 5 6	1 7 9
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																								+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma, metastatic, bone																X									
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma			X																						
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																					X				
Subcutaneous tissue, fibroma, multiple																				X					
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																X									
Nervous System																									
Brain	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																				+		+	+		
Spinal cord																				+		+	+		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear						+																			
Urinary System																									
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																						X		X	
Mesothelioma malignant																X									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Theophylline: 75 mg/kg (continued)

Number of Days on Study	706	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	733	733	733	733	734	734	734	
Carcass ID Number	18901	15102	15202	15902	16702	17002	17402	18202	18402	18502	19002	19402	15404	15505	15705	18001	18101	19101	15801	16801	18701	19901	16302	16402	16602	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+		+		+		+	+	+					+											9
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, bone																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibroadenoma					X																					1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma													X			X							X			4
Subcutaneous tissue, fibroma, multiple																										1
Subcutaneous tissue, lipoma															X											1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Peripheral nerve																										3
Spinal cord																										3
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma										X							X									2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X						X						X							X						6
Mesothelioma malignant						X							X													3

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Theophylline

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	7/50 (14%)	5/49 (10%)	7/50 (14%)	10/50 (20%)
Adjusted rate[b]	25.7%	15.1%	24.1%	36.1%
Terminal rate[c]	5/23 (22%)	4/32 (13%)	7/29 (24%)	7/24 (29%)
First incidence (days)	598	723	729 (T)	457
Life table test[d]	P=0.070	P=0.206N	P=0.467N	P=0.283
Logistic regression test[d]	P=0.043	P=0.320N	P=0.616N	P=0.163
Cochran-Armitage test[d]	P=0.143			
Fisher exact test[d]		P=0.394N	P=0.613N	P=0.298
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	8/50 (16%)	7/49 (14%)	7/50 (14%)	10/50 (20%)
Adjusted rate	29.8%	19.8%	24.1%	36.1%
Terminal rate	6/23 (26%)	5/32 (16%)	7/29 (24%)	7/24 (29%)
First incidence (days)	598	500	729 (T)	457
Life table test	P=0.159	P=0.292N	P=0.342N	P=0.384
Logistic regression test	P=0.115	P=0.480N	P=0.496N	P=0.234
Cochran-Armitage test	P=0.278			
Fisher exact test		P=0.517N	P=0.500N	P=0.398
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
Adjusted rate	10.9%	0.0%	3.4%	8.3%
Terminal rate	2/23 (9%)	0/33 (0%)	1/29 (3%)	2/24 (8%)
First incidence (days)	614	—[e]	729 (T)	729 (T)
Life table test	P=0.501	P=0.082N	P=0.254N	P=0.515N
Logistic regression test	P=0.473	P=0.116N	P=0.312N	P=0.585N
Cochran-Armitage test	P=0.563			
Fisher exact test		P=0.121N	P=0.309N	P=0.500N
Mammary Gland: Fibroadenoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	10.2%	6.1%	3.4%	4.2%
Terminal rate	0/23 (0%)	2/33 (6%)	1/29 (3%)	1/24 (4%)
First incidence (days)	670	729 (T)	729 (T)	729 (T)
Life table test	P=0.314N	P=0.382N	P=0.271N	P=0.334N
Logistic regression test	P=0.341N	P=0.466N	P=0.313N	P=0.372N
Cochran-Armitage test	P=0.263N			
Fisher exact test		P=0.500N	P=0.309N	P=0.309N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	10.2%	6.1%	3.4%	4.2%
Terminal rate	0/23 (0%)	2/33 (6%)	1/29 (3%)	1/24 (4%)
First incidence (days)	670	729 (T)	729 (T)	729 (T)
Life table test	P=0.314N	P=0.382N	P=0.271N	P=0.334N
Logistic regression test	P=0.341N	P=0.466N	P=0.313N	P=0.372N
Cochran-Armitage test	P=0.263N			
Fisher exact test		P=0.500N	P=0.309N	P=0.309N

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Pancreas: Adenoma				
Overall rate	10/50 (20%)	7/50 (14%)	4/50 (8%)	4/50 (8%)
Adjusted rate	38.6%	20.5%	13.8%	15.4%
Terminal rate	8/23 (35%)	6/33 (18%)	4/29 (14%)	3/24 (13%)
First incidence (days)	649	719	729 (T)	642
Life table test	P=0.115N	P=0.096N	P=0.029N	P=0.073N
Logistic regression test	P=0.156N	P=0.163N	P=0.061N	P=0.130N
Cochran-Armitage test	P=0.078N			
Fisher exact test		P=0.298N	P=0.074N	P=0.074N
Pancreatic Islets: Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	11.9%	3.0%	0.0%	0.0%
Terminal rate	1/23 (4%)	1/33 (3%)	0/29 (0%)	0/24 (0%)
First incidence (days)	726	729 (T)	—	—
Life table test	P=0.109N	P=0.203N	P=0.095N	P=0.126N
Logistic regression test	P=0.114N	P=0.211N	P=0.102N	P=0.132N
Cochran-Armitage test	P=0.097N			
Fisher exact test		P=0.309N	P=0.121N	P=0.121N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	19/50 (38%)	18/49 (37%)	16/50 (32%)	10/48 (21%)
Adjusted rate	55.2%	42.1%	42.7%	39.2%
Terminal rate	9/23 (39%)	9/33 (27%)	9/29 (31%)	9/24 (38%)
First incidence (days)	598	497	498	497
Life table test	P=0.102N	P=0.212N	P=0.236N	P=0.063N
Logistic regression test	P=0.070N	P=0.514N	P=0.364N	P=0.121N
Cochran-Armitage test	P=0.030N			
Fisher exact test		P=0.531N	P=0.338N	P=0.050N
Preputial Gland: Adenoma				
Overall rate	0/50 (0%)	2/49 (4%)	4/50 (8%)	3/50 (6%)
Adjusted rate	0.0%	5.6%	11.6%	12.5%
Terminal rate	0/23 (0%)	1/32 (3%)	2/29 (7%)	3/24 (13%)
First incidence (days)	—	666	498	729 (T)
Life table test	P=0.137	P=0.299	P=0.081	P=0.126
Logistic regression test	P=0.139	P=0.240	P=0.065	P=0.126
Cochran-Armitage test	P=0.200			
Fisher exact test		P=0.242	P=0.059	P=0.121
Preputial Gland: Carcinoma				
Overall rate	3/50 (6%)	2/49 (4%)	0/50 (0%)	4/50 (8%)
Adjusted rate	8.9%	6.3%	0.0%	13.7%
Terminal rate	0/23 (0%)	2/32 (6%)	0/29 (0%)	2/24 (8%)
First incidence (days)	572	729 (T)	—	498
Life table test	P=0.207	P=0.402N	P=0.125N	P=0.423
Logistic regression test	P=0.278	P=0.508N	P=0.111N	P=0.508
Cochran-Armitage test	P=0.291			
Fisher exact test		P=0.510N	P=0.121N	P=0.500

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Preputial Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	4/49 (8%)	4/50 (8%)	7/50 (14%)
Adjusted rate	8.9%	11.7%	11.6%	25.4%
Terminal rate	0/23 (0%)	3/32 (9%)	2/29 (7%)	5/24 (21%)
First incidence (days)	572	666	498	498
Life table test	P=0.056	P=0.622	P=0.533	P=0.131
Logistic regression test	P=0.076	P=0.494	P=0.516	P=0.118
Cochran-Armitage test	P=0.111			
Fisher exact test		P=0.489	P=0.500	P=0.159
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, or Basal Cell Adenoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	7.8%	9.1%	3.4%	0.0%
Terminal rate	1/23 (4%)	3/33 (9%)	1/29 (3%)	0/24 (0%)
First indidence (days)	700	729 (T)	729 (T)	—
Life table test	P=0.119N	P=0.654	P=0.438N	P=0.243N
Logistic regression test	P=0.134N	P=0.598	P=0.491N	P=0.275N
Cochran-Armitage test	P=0.104N			
Fisher exact test		P=0.500	P=0.500N	P=0.247N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	6/50 (12%)	1/50 (2%)	6/50 (12%)	5/50 (10%)
Adjusted rate	19.5%	3.0%	17.7%	18.1%
Terminal rate	1/23 (4%)	1/33 (3%)	3/29 (10%)	3/24 (13%)
First incidence (days)	614	729 (T)	558	595
Life table test	P=0.293	P=0.033N	P=0.550N	P=0.565N
Logistic regression test	P=0.312	P=0.052N	P=0.605	P=0.615N
Cochran-Armitage test	P=0.420			
Fisher exact test		P=0.056N	P=0.620N	P=0.500N
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Fibrous Histiocytoma				
Overall rate	6/50 (12%)	3/50 (6%)	6/50 (12%)	5/50 (10%)
Adjusted rate	19.5%	8.4%	17.7%	18.1%
Terminal rate	1/23 (4%)	2/33 (6%)	3/29 (10%)	3/24 (13%)
First incidence (days)	614	644	558	595
Life table test	P=0.406	P=0.150N	P=0.550N	P=0.565N
Logistic regression test	P=0.264	P=0.228N	P=0.605	P=0.615N
Cochran-Armitage test	P=0.548			
Fisher exact test		P=0.243N	P=0.620N	P=0.500N
Testes: Adenoma				
Overall rate	46/50 (92%)	42/50 (84%)	42/50 (84%)	44/50 (88%)
Adjusted rate	100.0%	97.6%	97.6%	97.8%
Terminal rate	23/23 (100%)	32/33 (97%)	28/29 (97%)	23/24 (96%)
First incidence (days)	529	372	425	387
Life table test	P=0.101	P=0.006N	P=0.087N	P=0.478
Logistic regression test	P=0.046	P=0.174N	P=0.348N	P=0.271
Cochran-Armitage test	P=0.539N			
Fisher exact test		P=0.178N	P=0.178N	P=0.370N

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Thyroid Gland (C-cell): Adenoma				
Overall rate	5/50 (10%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	15.4%	3.0%	8.1%	3.3%
Terminal rate	2/23 (9%)	1/33 (3%)	1/29 (3%)	0/24 (0%)
First incidence (days)	546	729 (T)	526	642
Life table test	P=0.243N	P=0.068N	P=0.344N	P=0.147N
Logistic regression test	P=0.150N	P=0.104N	P=0.322N	P=0.107N
Cochran-Armitage test	P=0.169N			
Fisher exact test		P=0.102N	P=0.357N	P=0.102N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	6/50 (12%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	19.5%	8.6%	8.1%	3.3%
Terminal rate	3/23 (13%)	2/33 (6%)	1/29 (3%)	0/24 (0%)
First incidence (days)	546	706	526	642
Life table test	P=0.110N	P=0.142N	P=0.224N	P=0.086N
Logistic regression test	P=0.071N	P=0.237N	P=0.224N	P=0.069N
Cochran-Armitage test	P=0.068N			
Fisher exact test		P=0.243N	P=0.243N	P=0.056N
All Organs: Mononuclear Cell Leukemia				
Overall rate	15/50 (30%)	5/50 (10%)	6/50 (12%)	6/50 (12%)
Adjusted rate	37.5%	14.0%	16.0%	21.8%
Terminal rate	3/23 (13%)	3/33 (9%)	0/29 (0%)	3/24 (13%)
First incidence (days)	529	706	572	643
Life table test	P=0.223N	P=0.007N	P=0.040N	P=0.072N
Logistic regression test	P=0.356N	P=0.013N	P=0.078N	P=0.199N
Cochran-Armitage test	P=0.096N			
Fisher exact test		P=0.011N	P=0.024N	P=0.024N
All Organs: Malignant Mesothelioma				
Overall rate	1/50 (2%)	2/50 (4%)	4/50 (8%)	3/50 (6%)
Adjusted rate	2.2%	5.1%	11.2%	11.0%
Terminal rate	0/23 (0%)	0/33 (0%)	2/29 (7%)	2/24 (8%)
First incidence (days)	572	624	498	552
Life table test	P=0.209	P=0.536	P=0.194	P=0.266
Logistic regression test	P=0.328	P=0.508	P=0.210	P=0.294
Cochran-Armitage test	P=0.304			
Fisher exact test		P=0.500	P=0.181	P=0.309
All Organs: Benign Neoplasms				
Overall rate	49/50 (98%)	49/50 (98%)	48/50 (96%)	46/50 (92%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	23/23 (100%)	33/33 (100%)	29/29 (100%)	24/24 (100%)
First incidence (days)	529	372	425	387
Life table test	P=0.193	P=0.042N	P=0.217N	P=0.510
Logistic regression test	P=0.544	P=0.420	P=0.730	P=0.647
Cochran-Armitage test	P=0.073N			
Fisher exact test		P=0.753N	P=0.500N	P=0.181N

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
All Organs: Malignant Neoplasms				
Overall rate	23/50 (46%)	18/50 (36%)	14/50 (28%)	14/50 (28%)
Adjusted rate	55.4%	42.7%	34.9%	43.3%
Terminal rate	6/23 (26%)	10/33 (30%)	4/29 (14%)	7/24 (29%)
First incidence (days)	529	372	498	306
Life table test	P=0.263N	P=0.083N	P=0.067N	P=0.151N
Logistic regression test	P=0.120N	P=0.217N	P=0.128N	P=0.071N
Cochran-Armitage test	P=0.067N			
Fisher exact test		P=0.208N	P=0.048N	P=0.048N
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	49/50 (98%)	48/50 (96%)	47/50 (94%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	23/23 (100%)	33/33 (100%)	29/29 (100%)	24/24 (100%)
First incidence (days)	529	372	425	306
Life table test	P=0.147	P=0.042N	P=0.217N	P=0.452
Logistic regression test	P=0.263	P=0.420	P=0.730	P=0.325
Cochran-Armitage test	P=0.181N			
Fisher exact test		P=0.753N	P=0.500N	P=0.309N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, lung, pancreas, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle 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 the vehicle controls and that dosed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE A4
Historical Incidence of Mononuclear Cell Leukemia in Vehicle Control Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence at Southern Research Institute	
Benzaldehyde	10/50
Furan	8/50
Furfural	13/50
Pentachloroanisole	23/50
Salicylazosulfapyridine	13/50
Overall Historical Incidence	
Total	237/972 (24.4%)
Standard deviation	10.0%
Range	10%-46%

[a] Data as of 12 May 1995. Includes data for lymphocytic, monocytic, mononuclear cell, or undifferentiated cell type leukemia

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1	1	1	
Moribund	21	11	18	13
Natural deaths	5	5	2	13
Survivors				
Terminal sacrifice	23	33	29	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Inflammation, focal		1 (2%)	1 (2%)	
Perforation	1 (2%)		1 (2%)	
Periesophageal tissue, inflammation, focal	1 (2%)			
Intestine large, rectum	(49)	(48)	(50)	(49)
Parasite metazoan		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)	1 (2%)	
Basophilic focus	25 (50%)	20 (40%)	26 (52%)	14 (28%)
Clear cell focus	4 (8%)	2 (4%)	1 (2%)	1 (2%)
Congestion	1 (2%)		2 (4%)	1 (2%)
Degeneration, cystic	2 (4%)			
Hemorrhage				1 (2%)
Hepatodiaphragmatic nodule	6 (12%)	2 (4%)	3 (6%)	4 (8%)
Hyperplasia, focal, histiocytic			1 (2%)	
Infiltration cellular, mixed cell	4 (8%)	3 (6%)	1 (2%)	1 (2%)
Mineralization, focal		1 (2%)		1 (2%)
Mixed cell focus	3 (6%)	3 (6%)	7 (14%)	3 (6%)
Necrosis, focal				2 (4%)
Vacuolization cytoplasmic	2 (4%)		1 (2%)	
Bile duct, hyperplasia	39 (78%)	39 (78%)	31 (62%)	29 (58%)
Mesentery	(16)	(16)	(17)	(27)
Accessory spleen			1 (6%)	
Artery, inflammation, chronic[b]	2 (4%)	2 (4%)	3 (6%)	15 (30%)
Fat, necrosis	15 (94%)	12 (75%)	15 (88%)	15 (56%)
Lymphatic, angiectasis				1 (4%)
Pancreas	(50)	(50)	(50)	(50)
Acinus, atrophy, focal	19 (38%)	13 (26%)	12 (24%)	9 (18%)
Acinus, hyperplasia	1 (2%)			
Acinus, hyperplasia, focal	9 (18%)	5 (10%)	2 (4%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum	1 (2%)			
Edema			1 (2%)	
Erosion	1 (2%)			
Inflammation, chronic	5 (10%)			3 (6%)
Ulcer	2 (4%)			
Epithelium, hyperplasia	6 (12%)			3 (6%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion
[b] Based on a special review of the mesenteric artery and associated tissues from all 50 animals in each dose group

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Alimentary System (continued)				
Stomach, glandular	(50)	(49)	(50)	(50)
Erosion	1 (2%)			
Inflammation, chronic	2 (4%)			
Mineralization		1 (2%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Fibrosis, focal				1 (2%)
Hypertrophy		1 (2%)		
Inflammation, chronic, focal			1 (2%)	
Mineralization		1 (2%)		1 (2%)
Pericardium, inflammation, suppurative			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	1 (2%)		3 (6%)	3 (6%)
Cytoplasmic alteration, focal	3 (6%)	1 (2%)	1 (2%)	
Hemorrhage	1 (2%)		1 (2%)	1 (2%)
Hyperplasia, focal	1 (2%)			
Infiltration cellular, focal, mixed cell				1 (2%)
Necrosis, focal	1 (2%)			
Adrenal medulla	(50)	(49)	(50)	(50)
Angiectasis			1 (2%)	
Hyperplasia	5 (10%)	9 (18%)	8 (16%)	4 (8%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			
Pituitary gland	(50)	(49)	(50)	(48)
Angiectasis	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Pars distalis, angiectasis			1 (2%)	
Pars distalis, cyst	2 (4%)	4 (8%)	3 (6%)	2 (4%)
Pars distalis, cytoplasmic alteration, focal	2 (4%)	3 (6%)	2 (4%)	2 (4%)
Pars distalis, degeneration, focal			1 (2%)	
Pars distalis, hyperplasia, focal	4 (8%)	3 (6%)	1 (2%)	3 (6%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	6 (12%)	5 (10%)	5 (10%)	2 (4%)
Follicle, cyst	2 (4%)	4 (8%)	2 (4%)	2 (4%)
Follicle, pigmentation, focal		1 (2%)	1 (2%)	
Follicular cell, hyperplasia			5 (10%)	
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)			
Inflammation, chronic		3 (6%)		1 (2%)

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Genital System (continued)				
Preputial gland	(50)	(49)	(50)	(50)
Cyst	1 (2%)			
Degeneration, cystic	10 (20%)	7 (14%)	10 (20%)	4 (8%)
Hyperplasia		3 (6%)		4 (8%)
Inflammation, chronic	5 (10%)	5 (10%)	8 (16%)	3 (6%)
Prostate	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Inflammation, chronic	30 (60%)	33 (66%)	31 (62%)	20 (40%)
Epithelium, hyperplasia, focal			5 (10%)	2 (4%)
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Germinal epithelium, degeneration	2 (4%)	4 (8%)	1 (2%)	
Interstitial cell, hyperplasia	2 (4%)	1 (2%)		1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Myelofibrosis	2 (4%)			
Necrosis, focal		1 (2%)		
Lymph node	(13)	(11)	(12)	(9)
Deep cervical, hyperplasia				1 (11%)
Iliac, ectasia		1 (9%)		
Iliac, hyperplasia			1 (8%)	
Inguinal, hyperplasia		1 (9%)	1 (8%)	
Inguinal, hyperplasia, lymphoid				1 (11%)
Inguinal, pigmentation		1 (9%)		
Mediastinal, ectasia		1 (9%)		
Mediastinal, hemorrhage	3 (23%)	3 (27%)	5 (42%)	5 (56%)
Mediastinal, hyperplasia, lymphoid	1 (8%)		1 (8%)	
Mediastinal, pigmentation	4 (31%)	3 (27%)	2 (17%)	2 (22%)
Pancreatic, ectasia			1 (8%)	
Pancreatic, hyperplasia, histiocytic	1 (8%)			
Pancreatic, pigmentation			1 (8%)	
Lymph node, mandibular	(48)	(49)	(50)	(50)
Congestion				1 (2%)
Ectasia			2 (4%)	1 (2%)
Hemorrhage		3 (6%)	6 (12%)	2 (4%)
Hyperplasia		1 (2%)	4 (8%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)			
Lymph node, mesenteric	(50)	(49)	(50)	(49)
Hyperplasia			1 (2%)	
Pigmentation	1 (2%)			
Spleen	(49)	(50)	(50)	(49)
Fibrosis, focal	4 (8%)	2 (4%)	2 (4%)	
Hematopoietic cell proliferation	4 (8%)	1 (2%)	2 (4%)	
Hemorrhage	1 (2%)			
Necrosis, focal		1 (2%)		
Thymus	(49)	(47)	(47)	(50)
Angiectasis			1 (2%)	3 (6%)
Cyst				2 (4%)
Hemorrhage	1 (2%)	1 (2%)		

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Integumentary System				
Mammary gland	(48)	(49)	(47)	(48)
Cyst			1 (2%)	1 (2%)
Ectasia	13 (27%)	10 (20%)	8 (17%)	6 (13%)
Hyperplasia	1 (2%)	1 (2%)		2 (4%)
Inflammation, chronic, focal			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Hyperkeratosis				1 (2%)
Inflammation, chronic, focal				2 (4%)
Ulcer				1 (2%)
Dermis, atrophy, focal		1 (2%)		
Epidermis, hyperplasia, focal		1 (2%)	1 (2%)	2 (4%)
Hair follicle, atrophy			1 (2%)	
Subcutaneous tissue, edema	1 (2%)			2 (4%)
Subcutaneous tissue, hemorrhage, focal	2 (4%)		1 (2%)	1 (2%)
Subcutaneous tissue, inflammation, chronic, focal				4 (8%)
Subcutaneous tissue, necrosis, focal			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Callus		1 (2%)		
Cranium, hemorrhage	1 (2%)			
Skeletal muscle	(4)	(1)	(1)	
Hemorrhage, focal	1 (25%)	1 (100%)		
Nervous System				
Brain	(50)	(49)	(50)	(49)
Atrophy, focal	10 (20%)	10 (20%)	8 (16%)	4 (8%)
Hemorrhage, focal	3 (6%)		3 (6%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	1 (2%)	1 (2%)		8 (16%)
Fibrosis, focal		1 (2%)		
Foreign body			1 (2%)	2 (4%)
Hemorrhage			2 (4%)	3 (6%)
Hyperplasia, histiocytic				1 (2%)
Inflammation, chronic		1 (2%)	1 (2%)	2 (4%)
Inflammation, suppurative	2 (4%)			
Necrosis, focal		1 (2%)		
Alveolar epithelium, hyperplasia	7 (14%)	2 (4%)	2 (4%)	1 (2%)
Interstitium, edema			1 (2%)	
Mediastinum, inflammation, acute			1 (2%)	
Serosa, foreign body			1 (2%)	1 (2%)
Serosa, inflammation			1 (2%)	
Nose	(50)	(49)	(50)	(50)
Inflammation, suppurative	5 (10%)	3 (6%)	7 (14%)	3 (6%)
Nasolacrimal duct, inflammation, suppurative		1 (2%)	1 (2%)	
Respiratory epithelium, hyperplasia, focal	1 (2%)	1 (2%)		

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Special Senses System				
Ear				(1)
External ear, inflammation, chronic, focal				1 (100%)
Eye	(8)	(7)	(15)	
Cataract	1 (13%)	1 (14%)		
Hemorrhage	2 (25%)			
Retinal detachment	1 (13%)			
Cornea, pigmentation	1 (13%)			
Retina, degeneration		1 (14%)		
Sclera, metaplasia, focal, osseous	1 (13%)	2 (29%)	8 (53%)	
Harderian gland	(1)			
Hypertrophy	1 (100%)			
Urinary System				
Kidney	(49)	(49)	(50)	(49)
Congestion	1 (2%)			
Cyst	2 (4%)		1 (2%)	1 (2%)
Infarct		1 (2%)		1 (2%)
Inflammation, suppurative		1 (2%)		
Nephropathy	46 (94%)	44 (90%)	49 (98%)	48 (98%)
Papilla, mineralization, focal		1 (2%)		
Papilla, necrosis		1 (2%)		
Renal tubule, accumulation, hyaline droplet	1 (2%)	1 (2%)		
Renal tubule, dilatation		2 (4%)		
Renal tubule, hyperplasia, focal			1 (2%)	
Renal tubule, pigmentation		1 (2%)	1 (2%)	
Ureter		(1)		
Inflammation, chronic		1 (100%)		
Transitional epithelium, hyperplasia		1 (100%)		
Urinary bladder	(50)	(50)	(50)	(49)
Inflammation, chronic		1 (2%)		

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF THEOPHYLLINE

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1		3
Moribund	17	12	14	7
Natural deaths	1	7	3	7
Survivors				
Died last week of study	1			
Terminal sacrifice	31	30	33	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(50)	(47)	(50)	(50)
Polyp adenomatous				1 (2%)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic		1 (2%)		
Hepatocellular adenoma			1 (2%)	
Sarcoma, metastatic, mesentery			1 (2%)	
Mesentery	(11)	(12)	(8)	(4)
Carcinoma, metastatic, islets, pancreatic		1 (8%)		
Fibroma				1 (25%)
Sarcoma			1 (13%)	
Oral mucosa			(1)	(2)
Hemangiosarcoma			1 (100%)	
Squamous cell papilloma				1 (50%)
Pancreas	(50)	(48)	(49)	(50)
Acinus, adenoma	1 (2%)	1 (2%)		2 (4%)
Acinus, sarcoma, metastatic, mesentery			1 (2%)	
Salivary glands	(50)	(49)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)		(1)	
Squamous cell carcinoma	1 (100%)		1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(49)	(50)	(49)
Pheochromocytoma complex	1 (2%)			
Pheochromocytoma benign	2 (4%)			1 (2%)
Islets, pancreatic	(50)	(49)	(50)	(50)
Adenoma		1 (2%)		1 (2%)
Carcinoma		1 (2%)		
Pituitary gland	(47)	(50)	(49)	(49)
Pars distalis, adenoma	22 (47%)	17 (34%)	17 (35%)	14 (29%)
Pars distalis, adenoma, multiple		1 (2%)		

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma		1 (2%)		
C-cell, adenoma	3 (6%)	7 (14%)	5 (10%)	6 (12%)
C-cell, carcinoma			1 (2%)	
Follicular cell, carcinoma	1 (2%)			1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(49)	(50)	(50)	(50)
Adenoma	4 (8%)	3 (6%)	1 (2%)	1 (2%)
Carcinoma		2 (4%)		
Ovary	(50)	(50)	(50)	(50)
Arrhenoblastoma benign	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Endometrium, carcinoma				1 (2%)
Endometrium, leiomyoma				1 (2%)
Endometrium, polyp stromal	9 (18%)	7 (14%)	11 (22%)	7 (14%)
Endometrium, polyp stromal, multiple			1 (2%)	
Endometrium, sarcoma stromal	1 (2%)	1 (2%)	3 (6%)	
Vagina	(1)			
Schwannoma malignant	1 (100%)			
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(50)
Lymph node	(9)	(10)	(9)	(8)
Mediastinal, sarcoma, metastatic, mesentery			1 (11%)	
Lymph node, mandibular	(49)	(48)	(50)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(48)	(50)	(50)
Thymus	(49)	(49)	(48)	(50)
Thymoma benign				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(49)
Carcinoma	1 (2%)	1 (2%)		
Fibroadenoma	17 (34%)	17 (34%)	11 (22%)	12 (24%)
Fibroadenoma, multiple	5 (10%)	2 (4%)	1 (2%)	
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma			1 (2%)	
Keratoacanthoma			1 (2%)	
Sebaceous gland, adenoma	1 (2%)			
Subcutaneous tissue, fibroma	2 (4%)	1 (2%)		1 (2%)
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	
Subcutaneous tissue, melanoma benign	1 (2%)			

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Musculoskeletal System				
Skeletal muscle			(1)	(1)
Sarcoma, metastatic, mesentery			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma malignant	1 (2%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)		1 (2%)
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Zymbal's gland	(1)			
Adenoma	1 (100%)			
Urinary System				
Kidney	(50)	(48)	(50)	(49)
Lipoma			1 (2%)	
Sarcoma	1 (2%)			
Urinary bladder	(50)	(49)	(50)	(50)
Leiomyosarcoma			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	10 (20%)	8 (16%)	9 (18%)	12 (24%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	44	41	40	36
Total primary neoplasms	87	72	69	65
Total animals with benign neoplasms	37	39	32	30
Total benign neoplasms	69	59	51	51
Total animals with malignant neoplasms	18	11	16	14
Total malignant neoplasms	18	13	18	14
Total animals with metastatic neoplasms		1	1	
Total metastatic neoplasms		2	4	

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: Vehicle Control

Number of Days on Study	3 9 9	4 4 1	4 9 8	4 9 8	5 2 4	5 3 3	5 4 5	5 7 6	5 8 0	6 3 0	6 6 7	6 7 7	6 7 0	6 7 0	6 7 8	6 9 1	7 1 9	7 2 0	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	2 2 0 9	2 0 1	2 3 2	2 3 5	2 3 9	2 2 1	2 4 3	2 4 4	2 1 6	2 1 0	2 0 8	2 1 9	2 1 7	2 1 8	2 3 4	2 5 0	2 0 9	2 4 7	2 0 7	2 0 2	2 0 3	2 0 4	2 0 5	2 0 6	2 1 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery		+				+	+									+			+					+	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+																								
Squamous cell carcinoma	X																								
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma complex																									
Pheochromocytoma benign								X								X								
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X					X	X		X	X	X			X			X			X	X		X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma															X										
Follicular cell, carcinoma																									
General Body System																									
Tissue NOS																								+	
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X									X				
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arrhenoblastoma benign																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal												X			X		X				X				
Endometrium, sarcoma stromal						X																			
Vagina										+															
Schwannoma malignant										X															

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	2 1 2	2 1 3	2 1 4	2 1 5	2 2 0	2 2 2	2 2 3	2 2 4	2 2 5	2 2 6	2 2 7	2 2 8	2 3 0	2 3 1	2 3 3	2 3 6	2 3 7	2 3 8	2 4 0	2 4 1	2 4 2	2 4 5	2 4 6	2 4 8	2 4 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery		+								+		+	+		+											11
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma																									X	1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Squamous cell carcinoma																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma complex				X																						1
Pheochromocytoma benign																										2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pars distalis, adenoma	X	X				X			X				X			X		X	X		X	X		X		22
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma										X	X															3
Follicular cell, carcinoma	X																									1
General Body System																										
Tissue NOS																										1
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma					X										X											4
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Arrhenoblastoma benign															X											1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal															X			X				X	X		X	9
Endometrium, sarcoma stromal																										1
Vagina																										1
Schwannoma malignant																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	3 9 9	4 4 1	4 9 8	4 9 8	5 2 4	5 3 3	5 4 5	5 7 6	5 8 0	6 3 0	6 6 7	6 6 7	6 7 0	6 7 0	6 8	6 9 1	7 1 9	7 2 0	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	2 2 0 9	2 0 1	2 3 2	2 3 5	2 3 9	2 4 1	2 4 3	2 4 4	2 1 6	2 1 0	2 0 8	2 1 9	2 1 7	2 1 8	2 3 4	2 5 0	2 0 9	2 4 7	2 0 7	2 0 2	2 0 3	2 0 4	2 0 5	2 0 6	2 1 1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+					+		+			+	+			+	+				+				
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma			X	X										X	X		X	X	X		X				
Fibroadenoma, multiple											X		X												
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sebaceous gland, adenoma																									
Subcutaneous tissue, fibroma												X													
Subcutaneous tissue, melanoma benign																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma malignant			X																						
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																						+			
Zymbal's gland																									
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									
Ureter																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X			X		X							X	X	X	X		X						

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	2 1 2	2 1 3	2 1 4	2 1 5	2 2 0	2 2 2	2 2 3	2 2 4	2 2 5	2 2 6	2 2 7	2 2 8	2 3 0	2 3 1	2 3 3	2 3 6	2 3 7	2 3 8	2 4 0	2 4 1	2 4 2	2 4 5	2 4 6	2 4 8	2 4 9	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node			+																							9
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																			X							1
Fibroadenoma			X	X		X		X		X	X							X			X				X	17
Fibroadenoma, multiple	X															X							X			5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sebaceous gland, adenoma									X																	1
Subcutaneous tissue, fibroma											X															2
Subcutaneous tissue, melanoma benign													X													1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma malignant																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																						+				2
Zymbal's gland																								+		1
Adenoma																								X		1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma							X																			1
Ureter												+														1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X	X															10

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg

Number of Days on Study	0 0 5	2 9 3	4 0 4	4 7 8	5 0 4	5 7 9	5 9 5	5 9 8	6 0 0	6 0 0	6 0 4	6 1 4	6 1 4	6 4 6	6 5 1	6 7 0	6 9 1	6 9 2	6 9 4	7 0 0	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	2 7 4	2 8 1	2 7 1	2 8 8	2 8 9	2 6 3	2 5 3	2 8 7	2 5 2	2 9 2	2 5 4	2 6 6	2 7 0	3 0 0	2 7 2	2 6 1	2 6 5	2 5 8	2 5 9	2 6 8	2 5 1	2 5 5	2 5 7	2 6 4	2 7 3
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, islets, pancreatic																X								
Mesentery							+							+		+		+				+		
Carcinoma, metastatic, islets, pancreatic																X								
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																								
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																								
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Carcinoma																X								
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma												X		X	X		X			X			X	X
Pars distalis, adenoma, multiple							X																	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																								
C-cell, adenoma																					X			
General Body System																								
Peritoneum																								
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X						X			X			
Carcinoma							X													X				
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal			X	X					X					X		X								
Endometrium, sarcoma stromal						X																		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	729	729	729	729	729	730	730	730	730	730	730	730	733	733	733	735	735	735	735	735	735	735	735	735	
Carcass ID Number	275	280	286	290	296	256	262	276	277	279	291	293	295	260	278	294	267	269	282	283	284	285	297	298	299	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, islets, pancreatic																										1
Mesentery			+			+			+		+		+	+				+								12
Carcinoma, metastatic, islets, pancreatic																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Acinus, adenoma																	X									1
Salivary glands	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+																									1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma															X											1
Carcinoma																										1
Parathyroid gland	+	+	+	+	M	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X	X	X			X			X	X					X	X					X				17
Pars distalis, adenoma, multiple																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, C-cell, adenoma					X																					1
C-cell, adenoma						X	X			X						X			X					X		7
General Body System																										
Peritoneum																	+									1
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										3
Carcinoma																										2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal									X														X			7
Endometrium, sarcoma stromal																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	0 0 5	2 9 3	4 0 4	4 7 8	5 0 4	5 7 9	5 9 5	5 9 8	6 0 0	6 0 0	6 1 4	6 1 4	6 1 4	6 4 6	6 5 1	6 7 0	6 9 1	6 9 2	6 9 4	7 0 0	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	2 7 4	2 8 1	2 7 1	2 8 8	2 8 9	2 6 3	2 5 3	2 8 7	2 5 2	2 9 2	2 5 4	2 6 6	2 7 0	3 0 0	2 7 2	2 6 1	2 6 5	2 5 8	2 5 9	2 6 8	2 5 1	2 5 5	2 5 7	2 6 4	2 7 3
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Lymph node		+					+				+							+	+				+	+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																	X								
Fibroadenoma											X	X	X			X				X		X	X	X	
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma										X															
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																									
Respiratory System																									
Larynx																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Lacrimal gland																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X												X	X	X	X				X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	729	729	729	729	729	730	730	730	730	730	730	730	730	733	733	733	735	735	735	735	735	735	735	735	735	
Carcass ID Number	27 5	28 0	28 6	29 0	29 6	25 6	26 2	27 6	27 7	27 9	29 1	29 3	29 5	26 0	27 8	29 4	26 7	26 9	28 2	28 3	28 4	28 5	29 7	29 8	29 9	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node					+				+				+													10
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Fibroadenoma	X			X			X	X						X				X					X	X	X	17
Fibroadenoma, multiple						X										X										2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																	+									1
Respiratory System																										
Larynx				+																						1
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																		X								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye					+					+								+								3
Lacrimal gland				+																						1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X												X									8

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 25 mg/kg

Number of Days on Study	4 9 7	4 9 8	5 2 0	5 3 1	5 4 1	5 5 1	6 0 1	6 1 7	6 4 2	6 4 9	6 6 1	6 6 3	6 7 0	6 7 1	6 7 4	7 1 9	7 2 3	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	3 1 6	3 4 5	3 4 1	3 4 4	3 2 3	3 3 2	3 2 7	3 3 7	3 2 2	3 3 0	3 1 8	3 0 9	3 3 4	3 1 4	3 0 1	3 2 1	3 4 6	3 0 2	3 0 3	3 0 4	3 0 8	3 1 9	3 2 0	3 2 9	3 3 6
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																		X							
Sarcoma, metastatic, mesentery			X																						
Mesentery	+		+		+											+		+	+					+	
Sarcoma			X																						
Oral mucosa									+																
Hemangiosarcoma									X																
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+
Acinus, sarcoma, metastatic, mesentery			X																						
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																	+								
Squamous cell carcinoma																	X								
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X	X			X		X			X					X				X					X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
C-cell, carcinoma																	X								
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X												
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal	X	X															X				X		X		
Endometrium, polyp stromal, multiple																								X	
Endometrium, sarcoma stromal					X					X											X				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 25 mg/kg (continued)

Number of Days on Study	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	3 3 8	3 4 7	3 0 5	3 0 7	3 1 0	3 1 1	3 1 2	3 1 3	3 1 5	3 2 4	3 2 5	3 3 9	3 4 0	3 4 0	3 0 6	3 2 6	3 2 8	3 4 2	3 4 3	3 1 7	3 3 1	3 3 3	3 3 5	3 4 8	3 4 9	3 5 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																										1
Sarcoma, metastatic, mesentery																										1
Mesentery																				+						8
Sarcoma																										1
Oral mucosa																										1
Hemangiosarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinus, sarcoma, metastatic, mesentery																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Squamous cell carcinoma																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	M	46
Pituitary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma		X				X	X		X									X	X		X	X		X		17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma	X						X		X									X	X							5
C-cell, carcinoma																										1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal			X												X	X		X	X					X		11
Endometrium, polyp stromal, multiple																										1
Endometrium, sarcoma stromal																										3

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 25 mg/kg (continued)

Number of Days on Study	4 9 7	4 9 8	5 2 0	5 3 1	5 4 1	5 5 1	6 0 1	6 1 7	6 4 2	6 4 9	6 6 1	6 6 3	6 7 0	6 7 1	6 7 4	7 1 9	7 2 3	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	3 1 6	3 4 5	3 4 1	3 4 4	3 2 3	3 3 2	3 2 7	3 3 7	3 2 2	3 3 0	3 1 8	3 0 9	3 3 4	3 1 4	3 0 1	3 2 1	3 4 6	3 0 2	3 0 3	3 0 4	3 0 8	3 1 9	3 2 0	3 2 9	3 3 6
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+								+	+		+	+	+			+		+				
Mediastinal, sarcoma, metastatic, mesentery			X																						
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma								X					X									X		X	
Fibroadenoma, multiple		X																							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Keratoacanthoma																								X	
Subcutaneous tissue, fibrous histiocytoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+																						
Sarcoma, metastatic, mesentery			X																						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve												+													
Spinal cord												+													
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Eye																			+		+				
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma	X																								
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X							X		X		X		X	X	X					

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 25 mg/kg (continued)

Number of Days on Study	729	729	730	730	730	730	730	730	730	730	730	730	730	733	733	733	733	733	735	735	735	735	735	735	735	
Carcass ID Number	338	347	305	307	310	311	312	313	315	324	325	339	340	306	326	328	342	343	317	331	333	335	348	349	350	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																			+							9
Mediastinal, sarcoma, metastatic, mesentery																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma		X	X	X			X				X	X		X												11
Fibroadenoma, multiple																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma		X																								1
Keratoacanthoma																										1
Subcutaneous tissue, fibrous histiocytoma																			X							1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Sarcoma, metastatic, mesentery																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear								+																		1
Eye	+		+		+									+	+	+		+								9
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lipoma																		X								1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X							X														9

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 75 mg/kg

Number of Days on Study	2 4 4	2 4 4	3 3 6	3 9 3	4 2 1	5 8 6	6 1 4	6 1 4	6 1 4	6 4 2	6 4 8	6 6 0	6 7 1	6 9 1	6 9 5	6 9 5	7 0 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	3 6 9	3 8 7	3 6 2	3 8 9	3 5 4	3 8 6	3 5 2	3 8 8	3 9 6	3 8 1	3 9 8	3 9 5	3 9 2	3 7 5	3 5 5	3 6 3	3 7 7	3 5 1	3 5 3	3 6 0	3 7 0	3 7 1	3 7 8	3 8 4	3 8 5
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																						X			
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery				+	+			+			+														
Fibroma											X														
Oral mucosa																									
Squamous cell papilloma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									X
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Pars distalis, adenoma						X						X	X								X		X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma											X										X	X			X
Follicular cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, carcinoma																								X	
Endometrium, leiomyoma																									
Endometrium, polyp stromal							X			X	X														

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 75 mg/kg (continued)

Number of Days on Study	729	730	730	730	730	730	730	733	733	733	733	733	733	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	39 7	35 6	35 7	37 2	77 3	39 0	99 1	55 8	55 9	57 4	77 6	79 3	99 4	66 1	66 4	66 5	66 6	66 7	66 8	67 9	68 0	68 2	68 3	69 9	40 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp adenomatous																										1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery																										4
Fibroma																										1
Oral mucosa																		+							+	2
Squamous cell papilloma																									X	1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma																							X			2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																X										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																					X					1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma			X	X		X					X				X	X						X		X	X	14
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma								X												X						6
Follicular cell, carcinoma																								X		1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																				X						1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, carcinoma																										1
Endometrium, leiomyoma																				X						1
Endometrium, polyp stromal		X				X										X	X									7

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 75 mg/kg (continued)

Number of Days on Study	2 4 4	2 4 4	3 3 6	3 9 3	4 2 1	5 8 6	6 1 4	6 1 4	6 1 4	6 4 2	6 4 8	6 6 0	6 7 1	6 9 1	6 9 5	6 9 5	7 0 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	3 6 9	3 8 7	3 6 2	3 8 9	3 5 4	3 8 6	3 5 2	3 8 8	3 9 6	3 8 1	3 9 8	3 9 5	3 9 2	3 7 5	3 5 5	3 6 3	3 7 7	3 5 1	3 5 3	3 6 0	3 7 0	3 7 1	3 7 8	3 8 4	3 8 5
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+			+			+				+					+					
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign										X															
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma				X					X						X		X								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma													X												
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve																+									
Spinal cord																+									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear															+	+									
Eye																+			+			+			
Urinary System																									
Kidney	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X			X		X					X	X	X		X						

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Theophylline: 75 mg/kg (continued)

Number of Days on Study	729	730	730	730	730	730	730	733	733	733	733	733	733	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	39 7	35 6	35 7	37 2	37 3	39 0	39 1	35 8	35 9	37 4	37 6	39 3	39 4	36 1	36 4	36 5	36 6	36 7	36 8	37 9	38 0	38 2	38 3	39 9	40 0	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node												+		+								+				8
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymoma benign																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibroadenoma			X			X		X			X										X		X	X	X	12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																			+							1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord												+														2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma												X														1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear		+																								3
Eye						+			+	+		+	+	+	+		+									11
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X	X									X										X				12

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Theophylline

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Adrenal Medulla: Benign or Complex Pheochromocytoma				
Overall rate[a]	3/50 (6%)	0/49 (0%)	0/50 (0%)	1/49 (2%)
Adjusted rate[b]	8.1%	0.0%	0.0%	3.0%
Terminal rate[c]	1/32 (3%)	0/30 (0%)	0/33 (0%)	1/33 (3%)
First incidence (days)	576	—[e]	—	729 (T)
Life table test[d]	P=0.433N	P=0.127N	P=0.121N	P=0.294N
Logistic regression test[d]	P=0.452N	P=0.122N	P=0.126N	P=0.313N
Cochran-Armitage test[d]	P=0.452N			
Fisher exact test[d]		P=0.125N	P=0.121N	P=0.316N
Clitoral Gland: Adenoma				
Overall rate	4/49 (8%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	11.7%	8.6%	2.6%	3.0%
Terminal rate	3/32 (9%)	1/30 (3%)	0/33 (0%)	1/33 (3%)
First incidence (days)	667	614	670	729 (T)
Life table test	P=0.127N	P=0.532N	P=0.179N	P=0.175N
Logistic regression test	P=0.131N	P=0.507N	P=0.167N	P=0.172N
Cochran-Armitage test	P=0.132N			
Fisher exact test		P=0.489N	P=0.175N	P=0.175N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	4/49 (8%)	5/50 (10%)	1/50 (2%)	1/50 (2%)
Adjusted rate	11.7%	13.6%	2.6%	3.0%
Terminal rate	3/32 (9%)	1/30 (3%)	0/33 (0%)	1/33 (3%)
First incidence (days)	667	595	670	729 (T)
Life table test	P=0.075N	P=0.470	P=0.179N	P=0.175N
Logistic regression test	P=0.076N	P=0.500	P=0.167N	P=0.172N
Cochran-Armitage test	P=0.077N			
Fisher exact test		P=0.513	P=0.175N	P=0.175N
Mammary Gland: Fibroadenoma				
Overall rate	22/50 (44%)	19/50 (38%)	12/50 (24%)	12/50 (24%)
Adjusted rate	54.4%	53.6%	32.2%	31.6%
Terminal rate	14/32 (44%)	14/30 (47%)	9/33 (27%)	8/33 (24%)
First incidence (days)	498	614	498	393
Life table test	P=0.022N	P=0.452N	P=0.033N	P=0.036N
Logistic regression test	P=0.023N	P=0.391N	P=0.025N	P=0.030N
Cochran-Armitage test	P=0.025N			
Fisher exact test		P=0.342N	P=0.028N	P=0.028N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	23/50 (46%)	20/50 (40%)	12/50 (24%)	12/50 (24%)
Adjusted rate	57.0%	55.0%	32.2%	31.6%
Terminal rate	15/32 (47%)	14/30 (47%)	9/33 (27%)	8/33 (24%)
First incidence (days)	498	614	498	393
Life table test	P=0.013N	P=0.456N	P=0.021N	P=0.024N
Logistic regression test	P=0.013N	P=0.394N	P=0.015N	P=0.019N
Cochran-Armitage test	P=0.015N			
Fisher exact test		P=0.343N	P=0.018N	P=0.018N

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	22/47 (47%)	18/50 (36%)	17/49 (35%)	14/49 (29%)
Adjusted rate	56.7%	49.2%	43.1%	38.2%
Terminal rate	15/31 (48%)	12/30 (40%)	11/32 (34%)	11/33 (33%)
First incidence (days)	498	595	498	586
Life table test	P=0.064N	P=0.335N	P=0.200N	P=0.060N
Logistic regression test	P=0.074N	P=0.237N	P=0.158N	P=0.059N
Cochran-Armitage test	P=0.073N			
Fisher exact test		P=0.191N	P=0.159N	P=0.051N
Thyroid Gland (C-cell): Adenoma				
Overall rate	3/50 (6%)	8/50 (16%)	5/50 (10%)	6/50 (12%)
Adjusted rate	8.9%	26.7%	15.2%	17.3%
Terminal rate	2/32 (6%)	8/30 (27%)	5/33 (15%)	5/33 (15%)
First incidence (days)	678	729 (T)	729 (T)	648
Life table test	P=0.486	P=0.079	P=0.369	P=0.258
Logistic regression test	P=0.474	P=0.073	P=0.368	P=0.243
Cochran-Armitage test	P=0.442			
Fisher exact test		P=0.100	P=0.357	P=0.243
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	8/50 (16%)	6/50 (12%)	6/50 (12%)
Adjusted rate	8.9%	26.7%	17.6%	17.3%
Terminal rate	2/32 (6%)	8/30 (27%)	5/33 (15%)	5/33 (15%)
First incidence (days)	678	729 (T)	723	648
Life table test	P=0.494	P=0.079	P=0.258	P=0.258
Logistic regression test	P=0.482	P=0.073	P=0.251	P=0.243
Cochran-Armitage test	P=0.449			
Fisher exact test		P=0.100	P=0.243	P=0.243
Uterus: Stromal Polyp				
Overall rate	9/50 (18%)	7/50 (14%)	12/50 (24%)	7/50 (14%)
Adjusted rate	25.2%	17.5%	32.2%	18.3%
Terminal rate	6/32 (19%)	2/30 (7%)	9/33 (27%)	4/33 (12%)
First incidence (days)	667	404	497	614
Life table test	P=0.370N	P=0.449N	P=0.340	P=0.379N
Logistic regression test	P=0.405N	P=0.388N	P=0.328	P=0.398N
Cochran-Armitage test	P=0.404N			
Fisher exact test		P=0.393N	P=0.312	P=0.393N
Uterus: Stromal Sarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.2%	2.2%	7.5%	0.0%
Terminal rate	0/32 (0%)	0/30 (0%)	1/33 (3%)	0/33 (0%)
First incidence (days)	533	579	541	—
Life table test	P=0.333N	P=0.753N	P=0.319	P=0.500N
Logistic regression test	P=0.328N	P=0.715N	P=0.239	P=0.423N
Cochran-Armitage test	P=0.339N			
Fisher exact test		P=0.753	P=0.309	P=0.500N

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	10/50 (20%)	8/50 (16%)	14/50 (28%)	7/50 (14%)
Adjusted rate	26.9%	19.3%	35.3%	18.3%
Terminal rate	6/32 (19%)	2/30 (7%)	9/33 (27%)	4/33 (12%)
First incidence (days)	533	404	497	614
Life table test	P=0.274N	P=0.449N	P=0.277	P=0.291N
Logistic regression test	P=0.293N	P=0.374N	P=0.241	P=0.304N
Cochran-Armitage test	P=0.295N			
Fisher exact test		P=0.398N	P=0.241	P=0.298N
All Organs: Mononuclear Cell Leukemia				
Overall rate	10/50 (20%)	8/50 (16%)	9/50 (18%)	12/50 (24%)
Adjusted rate	24.2%	22.0%	23.6%	30.0%
Terminal rate	3/32 (9%)	3/30 (10%)	5/33 (15%)	6/33 (18%)
First incidence (days)	441	478	541	421
Life table test	P=0.303	P=0.450N	P=0.482N	P=0.430
Logistic regression test	P=0.248	P=0.379N	P=0.527N	P=0.417
Cochran-Armitage test	P=0.247			
Fisher exact test		P=0.398N	P=0.500N	P=0.405
All Organs: Benign Neoplasms				
Overall rate	37/50 (74%)	39/50 (78%)	32/50 (64%)	30/50 (60%)
Adjusted rate	84.0%	88.5%	75.6%	69.4%
Terminal rate	25/32 (78%)	25/30 (83%)	23/33 (70%)	20/33 (61%)
First incidence (days)	498	404	497	393
Life table test	P=0.044N	P=0.297	P=0.199N	P=0.128N
Logistic regression test	P=0.036N	P=0.309	P=0.153N	P=0.112N
Cochran-Armitage test	P=0.037N			
Fisher exact test		P=0.408	P=0.194N	P=0.101N
All Organs: Malignant Neoplasms				
Overall rate	18/50 (36%)	11/50 (22%)	16/50 (32%)	14/50 (28%)
Adjusted rate	40.2%	27.9%	37.0%	35.2%
Terminal rate	7/32 (22%)	3/30 (10%)	7/33 (21%)	8/33 (24%)
First incidence (days)	399	478	497	421
Life table test	P=0.400N	P=0.154N	P=0.399N	P=0.274N
Logistic regression test	P=0.512	P=0.071N	P=0.482N	P=0.392N
Cochran-Armitage test	P=0.441N			
Fisher exact test		P=0.093N	P=0.417N	P=0.260N

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	44/50 (88%)	41/50 (82%)	40/50 (80%)	36/50 (72%)
Adjusted rate	88.0%	89.1%	84.9%	78.1%
Terminal rate	26/32 (81%)	25/30 (83%)	26/33 (79%)	23/33 (70%)
First incidence (days)	399	404	497	393
Life table test	P=0.077N	P=0.519N	P=0.265N	P=0.117N
Logistic regression test	P=0.100N	P=0.339N	P=0.219N	P=0.130N
Cochran-Armitage test	P=0.036N			
Fisher exact test		P=0.288N	P=0.207N	P=0.039N

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

TABLE B4
Historical Incidence of Mammary Gland Neoplasms in Vehicle Control Female F344/N Rats[a]

Study	Incidence in Controls		
	Fibroadenoma	Carcinoma	Fibroadenoma or Carcinoma
Historical Incidence at Southern Research Institute			
Benzaldehyde	28/50	1/50	28/50
Furan	15/50	1/50	15/50
Furfural	12/50	2/50	14/50
Pentachloroanisole	16/50	0/50	16/50
Salicylazosulfapyridine	22/50	2/50	24/50
Overall Historical Incidence			
Total	349/971 (35.9%)	24/971 (2.5%)	363/971 (37.4%)
Standard deviation	9.7%	2.2%	9.5%
Range	24%-56%	0%-6%	28%-56%

[a] Data as of 12 May 1995

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1		3
Moribund	17	12	14	7
Natural deaths	1	7	3	7
Survivors				
Died last week of study	1			
Terminal sacrifice	31	30	33	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation, focal		1 (2%)		
Perforation		1 (2%)		
Intestine large, colon	(50)	(47)	(50)	(50)
Parasite metazoan			4 (8%)	2 (4%)
Intestine large, rectum	(50)	(47)	(50)	(50)
Parasite metazoan	1 (2%)		4 (8%)	2 (4%)
Intestine large, cecum	(50)	(47)	(50)	(50)
Parasite metazoan				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)		2 (4%)	1 (2%)
Basophilic focus	40 (80%)	37 (74%)	31 (62%)	32 (64%)
Clear cell focus	3 (6%)			2 (4%)
Eosinophilic focus	2 (4%)			
Hemorrhage	1 (2%)			
Hepatodiaphragmatic nodule	5 (10%)	4 (8%)	7 (14%)	7 (14%)
Hyperplasia, focal, histiocytic	6 (12%)	2 (4%)	1 (2%)	3 (6%)
Hyperplasia, focal, lymphoid	4 (8%)	2 (4%)	1 (2%)	4 (8%)
Infiltration cellular, mixed cell	1 (2%)		5 (10%)	4 (8%)
Mineralization, focal			1 (2%)	
Mixed cell focus	2 (4%)	5 (10%)	5 (10%)	7 (14%)
Vacuolization cytoplasmic	4 (8%)			
Bile duct, hyperplasia	8 (16%)	7 (14%)	4 (8%)	7 (14%)
Centrilobular, degeneration			1 (2%)	
Centrilobular, necrosis	1 (2%)			
Mesentery	(11)	(12)	(8)	(4)
Accessory spleen	1 (9%)	1 (8%)		1 (25%)
Hemorrhage				1 (25%)
Infiltration cellular, focal, mixed cell	1 (9%)			
Inflammation, chronic	1 (9%)			
Necrosis, fatty		1 (8%)		
Artery, inflammation, chronic[b]	1 (2%)		2 (4%)	
Fat, necrosis	6 (55%)	9 (75%)	2 (25%)	
Vein, inflammation, chronic			1 (13%)	
Pancreas	(50)	(48)	(49)	(50)
Inflammation, chronic				1 (2%)
Acinus, atrophy, focal	3 (6%)	9 (19%)	10 (20%)	9 (18%)
Acinus, hyperplasia, focal		2 (4%)	1 (2%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion
[b] Based on a special review of the mesenteric artery and associated tissues from all 50 animals in each dose group.

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Alimentary System (continued)				
Salivary glands	(50)	(49)	(50)	(50)
Duct, cyst			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic	3 (6%)	1 (2%)		
Ulcer	1 (2%)			
Epithelium, hyperplasia	2 (4%)		1 (2%)	
Tooth		(1)		
Developmental malformation		1 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Thrombosis	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	1 (2%)	3 (6%)		2 (4%)
Angiectasis	1 (2%)			
Cytoplasmic alteration, focal	6 (12%)	2 (4%)	6 (12%)	5 (10%)
Hematopoietic cell proliferation			1 (2%)	
Hemorrhage	1 (2%)			1 (2%)
Vacuolization cytoplasmic				1 (2%)
Adrenal medulla	(50)	(49)	(50)	(49)
Hyperplasia	3 (6%)	4 (8%)	3 (6%)	5 (10%)
Pituitary gland	(47)	(50)	(49)	(49)
Angiectasis	6 (13%)	5 (10%)	6 (12%)	2 (4%)
Fibrosis, focal				1 (2%)
Metaplasia, focal, osseous		1 (2%)		
Pars distalis, angiectasis	1 (2%)			3 (6%)
Pars distalis, cyst	8 (17%)	14 (28%)	7 (14%)	7 (14%)
Pars distalis, cytoplasmic alteration, focal	2 (4%)	4 (8%)	2 (4%)	4 (8%)
Pars distalis, hyperplasia		1 (2%)		
Pars distalis, hyperplasia, focal	3 (6%)	1 (2%)	2 (4%)	3 (6%)
Pars distalis, necrosis				1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Ultimobranchial cyst			1 (2%)	
C-cell, hyperplasia	6 (12%)	3 (6%)	1 (2%)	5 (10%)
Follicle, cyst	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Follicular cell, hyperplasia			2 (4%)	1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(49)	(50)	(50)	(50)
Degeneration, cystic	8 (16%)	6 (12%)	8 (16%)	3 (6%)
Hyperplasia	3 (6%)	1 (2%)	3 (6%)	2 (4%)
Inflammation, chronic	2 (4%)		2 (4%)	2 (4%)
Ovary	(50)	(50)	(50)	(50)
Cyst	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Bilateral, cyst		1 (2%)	1 (2%)	

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Genital System (continued)				
Uterus	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Hydrometra	1 (2%)	3 (6%)	2 (4%)	3 (6%)
Hyperplasia, cystic	1 (2%)			
Cervix, hypertrophy				2 (4%)
Endometrium, hyperplasia, cystic	8 (16%)	7 (14%)	8 (16%)	11 (22%)
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(50)
Depletion cellular	1 (2%)	1 (2%)		
Hyperplasia		1 (2%)		
Hyperplasia, focal, histiocytic			1 (2%)	4 (8%)
Myelofibrosis		1 (2%)	1 (2%)	
Lymph node	(9)	(10)	(9)	(8)
Iliac, ectasia	1 (11%)			
Inguinal, pigmentation		1 (10%)		
Mediastinal, ectasia	1 (11%)			
Mediastinal, hemorrhage			1 (11%)	2 (25%)
Mediastinal, pigmentation	4 (44%)	3 (30%)	1 (11%)	2 (25%)
Pancreatic, ectasia			1 (11%)	
Pancreatic, pigmentation			1 (11%)	
Renal, hyperplasia		1 (10%)		
Lymph node, mandibular	(49)	(48)	(50)	(50)
Congestion				2 (4%)
Ectasia			1 (2%)	1 (2%)
Hemorrhage			3 (6%)	
Hyperplasia	1 (2%)			
Hyperplasia, lymphoid			2 (4%)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Hyperplasia, lymphoid			1 (2%)	
Pigmentation		2 (4%)	1 (2%)	1 (2%)
Spleen	(50)	(48)	(50)	(50)
Hematopoietic cell proliferation	5 (10%)	7 (15%)	11 (22%)	3 (6%)
Hemorrhage				1 (2%)
Pigmentation			2 (4%)	
Thymus	(49)	(49)	(48)	(50)
Angiectasis				1 (2%)
Cyst	1 (2%)	1 (2%)		2 (4%)
Hemorrhage				1 (2%)
Hyperplasia, lymphoid				1 (2%)

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Integumentary System				
Mammary gland	(50)	(50)	(50)	(49)
Cyst	1 (2%)			
Ectasia	26 (52%)	23 (46%)	23 (46%)	20 (41%)
Hyperplasia	5 (10%)	3 (6%)	4 (8%)	6 (12%)
Skin	(50)	(50)	(50)	(50)
Inflammation, chronic, focal	2 (4%)	1 (2%)	2 (4%)	
Ulcer	1 (2%)	3 (6%)	1 (2%)	
Subcutaneous tissue, fibrosis, focal	1 (2%)			
Subcutaneous tissue, hemorrhage, focal	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis	5 (10%)	3 (6%)	2 (4%)	2 (4%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Atrophy, focal	10 (20%)	7 (14%)	7 (14%)	8 (16%)
Hemorrhage, focal	3 (6%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion		2 (4%)	1 (2%)	5 (10%)
Hemorrhage		1 (2%)	1 (2%)	
Hyperplasia, histiocytic			1 (2%)	1 (2%)
Inflammation, chronic		1 (2%)		
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Interstitium, edema			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Foreign body	1 (2%)			
Inflammation, suppurative	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Mucosa, glands, dilatation, focal		1 (2%)		
Nasolacrimal duct, inflammation, suppurative	1 (2%)	1 (2%)		1 (2%)
Special Senses System				
Eye	(2)	(3)	(9)	(11)
Cataract				2 (18%)
Inflammation, chronic				1 (9%)
Cornea, inflammation, chronic				2 (18%)
Cornea, pigmentation				1 (9%)
Retina, degeneration				1 (9%)
Sclera, metaplasia, focal, osseous		1 (33%)	3 (33%)	

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Urinary System				
Kidney	(50)	(48)	(50)	(49)
Angiectasis				1 (2%)
Cyst		1 (2%)	2 (4%)	
Fibrosis, focal				1 (2%)
Inflammation, chronic			1 (2%)	
Inflammation, suppurative				1 (2%)
Nephropathy	43 (86%)	36 (75%)	41 (82%)	41 (84%)
Papilla, mineralization, focal		1 (2%)		
Pelvis, dilatation		1 (2%)		
Pelvis, mineralization			2 (4%)	
Pelvis, transitional epithelium, hyperplasia	1 (2%)	1 (2%)	2 (4%)	
Renal tubule, pigmentation		1 (2%)		
Ureter	(1)			
Inflammation, chronic	1 (100%)			
Transitional epithelium, hyperplasia	1 (100%)			
Urinary bladder	(50)	(49)	(50)	(50)
Calculus, gross observation	1 (2%)		1 (2%)	
Calculus, microscopic observation only	1 (2%)			
Inflammation, chronic	1 (2%)			
Transitional epithelium, hyperplasia	1 (2%)		1 (2%)	

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF THEOPHYLLINE

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

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	9	2	9
Natural deaths	5	6	4	15
Survivors				
Terminal sacrifice	36	35	44	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(45)	(46)	(47)	(42)
Adenoma		1 (2%)		
Intestine small, duodenum	(48)	(49)	(49)	(41)
Leiomyosarcoma	1 (2%)			
Intestine small, jejunum	(48)	(47)	(47)	(41)
Polyp adenomatous	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)	1 (2%)	1 (2%)	
Hemangiosarcoma, multiple		2 (4%)		
Hepatocellular carcinoma	12 (24%)	13 (26%)	10 (20%)	2 (4%)
Hepatocellular carcinoma, multiple	7 (14%)	1 (2%)	2 (4%)	
Hepatocellular adenoma	14 (28%)	12 (24%)	10 (20%)	2 (4%)
Hepatocellular adenoma, multiple	7 (14%)	6 (12%)	2 (4%)	
Histiocytic sarcoma	1 (2%)	1 (2%)	1 (2%)	
Mesentery	(10)	(4)	(2)	
Histiocytic sarcoma	1 (10%)	1 (25%)		
Sarcoma		1 (25%)		
Pancreas	(50)	(49)	(49)	(50)
Stomach, forestomach	(50)	(50)	(50)	(49)
Squamous cell carcinoma			1 (2%)	
Squamous cell papilloma	1 (2%)	4 (8%)		1 (2%)
Stomach, glandular	(50)	(49)	(49)	(43)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Capsule, adenoma	4 (8%)	2 (4%)	1 (2%)	1 (2%)
Adrenal medulla	(49)	(50)	(49)	(49)
Pheochromocytoma benign		1 (2%)		
Islets, pancreatic	(50)	(49)	(50)	(50)
Adenoma			1 (2%)	
Pituitary gland	(49)	(50)	(48)	(44)
Pars intermedia, adenoma			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma		1 (2%)		
Follicular cell, adenoma		1 (2%)		2 (4%)
Follicular cell, carcinoma		1 (2%)		

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

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
General Body System				
Tissue NOS	(1)			
Genital System				
Epididymis	(49)	(50)	(50)	(50)
Preputial gland	(49)	(48)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Prostate	(50)	(49)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Hemangiosarcoma	2 (4%)	1 (2%)		
Histiocytic sarcoma		1 (2%)	1 (2%)	
Lymph node	(5)	(6)	(5)	(3)
Mediastinal, carcinoma, metastatic, lung	1 (20%)			
Pancreatic, histiocytic sarcoma	1 (20%)			
Renal, histiocytic sarcoma			1 (20%)	
Lymph node, mandibular	(42)	(47)	(47)	(47)
Histiocytic sarcoma			1 (2%)	
Lymph node, mesenteric	(50)	(46)	(50)	(48)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Spleen	(50)	(49)	(50)	(49)
Hemangiosarcoma	2 (4%)	1 (2%)		
Histiocytic sarcoma	1 (2%)		1 (2%)	
Thymus	(43)	(42)	(44)	(46)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrosarcoma		1 (2%)	1 (2%)	
Subcutaneous tissue, hemangiosarcoma		1 (2%)		
Subcutaneous tissue, sarcoma		1 (2%)		
Musculoskeletal System				
Skeletal muscle	(1)			
Hemangiosarcoma	1 (100%)			
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	8 (16%)	6 (12%)	8 (16%)	3 (6%)
Alveolar/bronchiolar adenoma, multiple		2 (4%)	2 (4%)	1 (2%)
Alveolar/bronchiolar carcinoma	6 (12%)	1 (2%)	5 (10%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	6 (12%)	1 (2%)		
Histiocytic sarcoma	1 (2%)	1 (2%)		
Sarcoma	1 (2%)			
Nose	(50)	(50)	(50)	(50)

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

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Special Senses System				
Harderian gland	(1)	(4)	(2)	(2)
Adenoma	1 (100%)	3 (75%)	2 (100%)	2 (100%)
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Carcinoma, metastatic, lung	1 (2%)			
Renal tubule, adenoma		1 (2%)		
Urinary bladder	(50)	(49)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	1 (2%)	
Lymphoma malignant	5 (10%)	3 (6%)	5 (10%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	44	42	38	13
Total primary neoplasms	76	69	54	16
Total animals with benign neoplasms	29	29	22	9
Total benign neoplasms	36	40	27	12
Total animals with malignant neoplasms	32	24	25	4
Total malignant neoplasms	40	29	27	4
Total animals with metastatic neoplasms	7	1		
Total metastatic neoplasms	9	1		

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: Vehicle Control

Number of Days on Study	4 3 8	4 8 5	6 0 5	6 2 7	6 2 7	6 3 5	6 3 5	6 7 6	7 0 0	7 0 0	7 0 1	7 0 3	7 0 4	7 1 1	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 0 8	0 0 3	0 1 6	0 1 3	0 4 7	0 1 1	0 2 7	0 1 7	0 1 4	0 3 2	0 3 6	0 2 0	0 0 5	0 2 4	0 0 1	0 0 2	0 0 4	0 0 6	0 0 7	0 0 9	0 1 0	0 1 2	0 1 5	0 1 8	0 1 9
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma													X												
Intestine small, jejunum	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous			X																						
Intestine small, ileum	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																		
Hepatocellular carcinoma				X				X	X		X												X		
Hepatocellular carcinoma, multiple		X	X		X	X																			
Hepatocellular adenoma			X														X	X					X		
Hepatocellular adenoma, multiple							X					X	X		X			X	X						
Histiocytic sarcoma		X																							
Mesentery		+			+	+			+			+		+							+	+			
Histiocytic sarcoma		X																							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth							+							+					+		+			+	
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, kidney										X															
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																	X								
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
Tissue NOS	+																								
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Penis											+														
Preputial gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	02 1	02 2	02 3	02 5	02 6	02 8	02 9	03 0	03 1	03 3	03 4	03 5	03 7	03 8	03 9	04 0	04 1	04 2	04 3	04 4	04 5	04 6	04 8	04 9	05 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leiomyosarcoma																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Polyp adenomatous																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma				X																						2
Hepatocellular carcinoma						X				X	X				X				X				X		X	12
Hepatocellular carcinoma, multiple	X							X																X		7
Hepatocellular adenoma						X		X	X	X		X	X					X		X	X		X			14
Hepatocellular adenoma, multiple														X												7
Histiocytic sarcoma																										1
Mesentery			+	+																						10
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma	X																									1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+													+						+	+				+	10
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, kidney																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma					X				X											X						4
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
Tissue NOS																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Penis																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	4 3 8	4 8 5	6 0 5	6 2 7	6 2 7	6 3 5	6 3 5	6 7 6	7 0 0	7 0 0	7 0 1	7 0 3	7 0 4	7 1 1	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 0 0 8	0 0 3	0 1 6	1 1 3	0 4 7	1 1 1	0 2 7	1 1 7	1 1 4	0 3 2	3 3 6	2 0 0	0 5	2 4	0 1	0 2	0 4	0 6	0 7	0 9	1 0	1 2	1 5	1 8	1 9
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																		
Lymph node		+								+			+						+		+				
Mediastinal, carcinoma, metastatic, lung										X															
Pancreatic, histiocytic sarcoma		X																							
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																		
Histiocytic sarcoma		X																							
Thymus	+	M	+	+	M	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle							+																		
Hemangiosarcoma							X																		
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve												+													
Spinal cord												+													
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																							X		
Alveolar/bronchiolar carcinoma										X						X									
Hepatocellular carcinoma, metastatic, liver			X		X	X		X																	
Histiocytic sarcoma		X																							
Sarcoma																						X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland										+															
Adenoma										X															
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, lung										X															
Urethra																								+	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Lymphoma malignant	X	X																		X	X				

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 2 1	0 2 2	0 2 3	0 2 5	0 2 6	0 2 8	0 2 9	0 3 0	0 3 1	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 2	0 4 3	0 4 4	0 4 5	0 4 6	0 4 8	0 4 9	0 5 0	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma							X																			2
Lymph node																										5
Mediastinal, carcinoma, metastatic, lung																										1
Pancreatic, histiocytic sarcoma																										1
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	42
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma							X																			2
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	43
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Hemangiosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X		X			X					X			X		X				X					8
Alveolar/bronchiolar carcinoma										X			X								X				X	6
Hepatocellular carcinoma, metastatic, liver															X				X							6
Histiocytic sarcoma																										1
Sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland																										1
Adenoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, lung																										1
Urethra	+																								+	3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant												X														5

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 15 mg/kg

Number of Days on Study	4 2 1	4 6 4	5 1 1	5 2 1	5 2 2	5 5 4	5 6 2	5 6 9	6 2 7	6 2 7	6 3 3	6 3 5	6 9 8	7 0 6	7 1 1	7 2 5	7 2 5	7 2 5	7 2 5	7 2 5	7 2 5	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 0 9 5	0 0 9 9	0 0 8 1	0 0 8 7	0 0 5 8	0 0 9 1	0 0 5 4	0 0 6 5	0 0 5 1	0 0 5 6	0 0 9 3	1 0 0 0	0 0 7 7	0 0 9 8	0 0 9 7	0 0 5 2	0 0 6 8	0 0 7 0	0 0 7 3	0 0 8 2	0 0 8 9	0 0 9 6	0 0 5 3	0 0 5 7	0 0 6 0	0 0 6 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	A	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Intestine large, colon	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	M	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hemangiosarcoma, multiple															X										
Hepatocellular carcinoma				X	X			X	X				X	X			X							X	
Hepatocellular carcinoma, multiple		X																							
Hepatocellular adenoma				X						X							X	X		X					
Hepatocellular adenoma, multiple																			X				X		X
Histiocytic sarcoma								X																	
Mesentery	+							+												+				+	
Histiocytic sarcoma								X																	
Sarcoma	X																								
Pancreas	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																							X		
Stomach, glandular	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth										+			+												
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																		X							
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
Follicular cell, adenoma																									
Follicular cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	M	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Histiocytic sarcoma								X																	
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 15 mg/kg (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	00662	00663	00666	00774	00775	00776	00883	00884	00885	00886	00888	00890	00894	00895	00959	00964	00967	00969	00971	00972	00978	00979	00980	00989	00992	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenoma	X																									1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																		X								1
Hemangiosarcoma, multiple																							X			2
Hepatocellular carcinoma		X					X	X								X									X	13
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma	X			X		X		X				X											X	X		12
Hepatocellular adenoma, multiple							X								X	X										6
Histiocytic sarcoma																										1
Mesentery																										4
Histiocytic sarcoma																										1
Sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma									X							X							X			4
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth									+			+						+								5
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma																						X				2
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																									X	1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma			X																							1
Follicular cell, adenoma												X														1
Follicular cell, carcinoma										X																1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 15 mg/kg (continued)

Number of Days on Study	4 2 1	4 6 4	5 1 1	5 2 1	5 2 2	5 5 4	5 6 2	5 6 9	6 2 7	6 2 7	6 3 3	6 3 5	6 9 8	7 0 6	7 1 1	7 2 5	7 2 5	7 2 5	7 2 5	7 2 5	7 2 5	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 9 5	0 9 9	0 8 1	0 8 7	0 5 8	0 9 1	0 5 4	0 6 5	0 5 1	0 5 6	0 9 3	1 0 0	0 7 7	0 9 8	0 9 7	0 5 2	0 6 8	0 7 0	0 7 3	0 8 2	0 9 6	0 5 3	0 5 7	0 6 0	0 6 1
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma			X																					
Histiocytic sarcoma								X																
Lymph node	+												+				+						+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	+	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Histiocytic sarcoma								X																
Spleen	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																								
Thymus	+	+	A	+	+	+	+	+	M	I	+	M	+	+	+	+	+	+	I	+	+	+	+	+
Integumentary System																								
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma													X											
Subcutaneous tissue, hemangiosarcoma			X																					
Subcutaneous tissue, sarcoma										X														
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X						X			X	X			
Alveolar/bronchiolar adenoma, multiple																								
Alveolar/bronchiolar carcinoma																								
Hepatocellular carcinoma, metastatic, liver																							X	
Histiocytic sarcoma								X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Eye														+										
Harderian gland																								
Adenoma																								
Urinary System																								
Kidney	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																		X						
Urethra			+																					
Urinary bladder	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																
Lymphoma malignant							X																X	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 15 mg/kg (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	00662	00663	00666	00674	00675	00676	00683	00684	00685	00686	00688	00690	00694	00695	00659	00664	00667	00669	00671	00672	00678	00679	00680	00689	00692		Total Tissues/ Tumors
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Hemangiosarcoma																											1
Histiocytic sarcoma																											1
Lymph node													+														6
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Histiocytic sarcoma																											1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Hemangiosarcoma								X																			1
Thymus	+	M	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+		42
Integumentary System																											
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Subcutaneous tissue, fibrosarcoma																											1
Subcutaneous tissue, hemangiosarcoma																											1
Subcutaneous tissue, sarcoma																											1
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar adenoma														X						X							6
Alveolar/bronchiolar adenoma, multiple			X																X								2
Alveolar/bronchiolar carcinoma						X																					1
Hepatocellular carcinoma, metastatic, liver																											1
Histiocytic sarcoma																											1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Special Senses System																											
Eye																											1
Harderian gland					+					+				+			+										4
Adenoma										X				X			X										3
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Renal tubule, adenoma																											1
Urethra									+			+															3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Histiocytic sarcoma																											1
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Histiocytic sarcoma																											1
Lymphoma malignant																									X		3

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 50 mg/kg

Number of Days on Study	5 5 6	5 7 7	6 2 0	6 6 8	6 7 2	7 0 0	7 2 5	7 2 5	7 2 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 1 3	1 3 9	1 3 0	1 3 2	1 4 7	1 3 6	1 1 1	1 2 3	1 4 8	1 0 1	1 0 2	1 0 3	1 0 4	1 0 5	1 1 2	1 1 4	1 1 5	1 2 1	1 2 2	1 2 4	1 2 5	1 3 1	1 3 3	1 3 4	1 3 5
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hepatocellular carcinoma	X					X						X	X					X	X				X		
Hepatocellular carcinoma, multiple																				X					
Hepatocellular adenoma				X							X				X										X
Hepatocellular adenoma, multiple												X												X	
Histiocytic sarcoma								X																	
Mesentery					+					+															
Pancreas	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																									
Adrenal medulla	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Pituitary gland	+	+	+	+	+	I	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 50 mg/kg (continued)

Number of Days on Study	729	729	729	729	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	14041	14042	14043	14044	14046	14047	14048	14049	14050	14116	14117	14118	14119	14120	14126	14127	14128	14129	14137	14138	14140	14145	14146	14149	14150	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																									X	1
Hepatocellular carcinoma											X												X		X	10
Hepatocellular carcinoma, multiple							X																			2
Hepatocellular adenoma	X												X					X	X			X	X			10
Hepatocellular adenoma, multiple																										2
Histiocytic sarcoma																										1
Mesentery																										2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																		X								1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma																				X						1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																					X					1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars intermedia, adenoma				X																						1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma									X																	1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 50 mg/kg (continued)

Number of Days on Study	556	577	620	668	672	700	725	725	725	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	11313	13939	13300	13302	14147	13316	11761	12353	14248	14001	10912	10003	10004	10005	11012	11414	11515	12121	12224	12425	12955	13313	13434	13435
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																
Lymph node				+	+			+																
Renal, histiocytic sarcoma								X																
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Histiocytic sarcoma								X																
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																
Thymus	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	M	M	+	+	+	+
Integumentary System																								
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma		X																						
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord																								
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma												X						X		X				X
Alveolar/bronchiolar adenoma, multiple																					X			
Alveolar/bronchiolar carcinoma			X													X			X			X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Harderian gland																								
Adenoma																								
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra																			+					
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																
Lymphoma malignant				X	X																			

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 50 mg/kg (continued)

Number of Days on Study	729	729	729	729	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	14 1	14 2	14 3	14 4	10 6	10 7	10 8	10 9	11 0	11 6	11 7	11 8	11 9	12 0	12 6	12 7	12 8	12 9	13 7	13 8	14 0	14 5	14 6	14 9	15 0	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node		+				+																				5
Renal, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	44
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																	+									1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X			X						X				X							8
Alveolar/bronchiolar adenoma, multiple																	X									2
Alveolar/bronchiolar carcinoma	X																									5
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland				+													+									2
Adenoma				X													X									2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urethra																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant		X	X			X																				5

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 150 mg/kg

Number of Days on Study	0 1 6	0 3 2	0 9 5	1 2 1	1 8 4	2 1 1	2 1 2	2 8 2	3 3 1	3 3 2	3 3 4	3 5 2	3 5 9	3 5 9	3 8 2	4 0 5	4 0 7	4 4 2	4 4 9	4 8 1	5 9 8	6 3 6	6 3 6	7 0 3	7 2 5
Carcass ID Number	1 5 2	1 5 7	1 9 7	1 9 5	1 5 8	1 7 2	1 7 9	1 9 6	1 7 6	1 9 2	1 6 4	1 8 2	1 8 7	1 9 1	1 5 3	1 7 4	1 6 9	1 5 4	1 7 5	1 6 3	1 9 9	1 9 3	1 9 4	1 8 8	1 6 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	M	+	+	A	+	+	+	+	+	+	+	A	A	+	+	+	+	+	A	A	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	A	+	+	+	+	A	+	A	+	+	+	+	A	A	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	A	+	+	+	+	A	A	+	+
Intestine large, cecum	+	+	+	+	+	+	A	+	+	A	+	+	+	+	A	A	+	+	+	A	+	A	A	+	+
Intestine small, duodenum	A	+	+	+	A	+	+	+	+	A	+	+	+	+	A	+	A	A	+	A	+	A	A	+	+
Intestine small, jejunum	A	+	+	+	A	+	+	+	A	A	+	+	+	+	A	A	+	+	+	A	+	A	A	+	+
Intestine small, ileum	A	+	+	+	A	+	A	+	A	A	+	+	+	+	A	A	A	A	+	A	+	A	A	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																						X			
Hepatocellular adenoma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									X
Stomach, glandular	A	+	+	+	+	+	+	+	+	A	+	+	+	+	A	A	+	+	+	A	+	A	A	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Capsule, adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	I	+	+	+	+	+	M	I	M	+	+	+	+	M	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																							X		
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Lymph node																+									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 150 mg/kg (continued)

Number of Days on Study	725	725	725	726	726	726	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	
Carcass ID Number	17303	18606	20000	15505	18101	18404	15101	16101	16505	16606	16707	16808	17000	17101	17707	17808	18003	18503	15606	15909	16000	18909	19000	19808	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	42
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	38
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																				X					2
Hepatocellular adenoma							X												X						2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma																									1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Capsule, adenoma																						X			1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	44
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																						X			2
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+																			+					3
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	46

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 150 mg/kg (continued)

Number of Days on Study	0 1 6	0 3 2	0 9 5	1 2 1	1 8 4	2 1 1	2 1 2	2 8 2	3 3 1	3 3 2	3 3 4	3 5 2	3 5 9	3 5 9	3 8 2	4 0 5	4 0 7	4 4 2	4 4 9	4 8 1	5 9 8	6 3 6	6 3 6	7 0 3	7 2 5
Carcass ID Number	1 5 2	1 5 7	1 9 7	1 9 5	1 5 8	1 7 2	1 7 9	1 9 6	1 7 6	1 9 2	1 6 4	1 8 2	1 8 7	1 9 1	1 5 3	1 7 4	1 6 9	1 5 4	1 7 5	1 6 3	1 9 9	1 9 3	1 9 4	1 8 8	1 6 2
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																							X		
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland																	+								
Adenoma																	X								
Lacrimal gland		+																							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ureter										+															
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant															X										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Theophylline: 150 mg/kg (continued)

Number of Days on Study	725	725	725	726	726	726	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	
Carcass ID Number	17373	18686	20005	15055	18811	18844	15151	16161	16565	16667	16687	16688	17070	17171	17717	17878	18080	18383	18585	15656	15959	16090	18989	19090	19898		Total Tissues/ Tumors
Integumentary System																											
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X																X								3
Alveolar/bronchiolar adenoma, multiple								X																			1
Alveolar/bronchiolar carcinoma														X													1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																											
Harderian gland																				+							2
Adenoma																				X							2
Lacrimal gland																											1
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ureter																											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant																											1

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Theophylline

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	4/50 (8%)	2/50 (4%)	1/50 (2%)	1/49 (2%)
Adjusted rate[b]	11.1%	5.7%	2.3%	3.8%
Terminal rate[c]	4/36 (11%)	2/35 (6%)	1/44 (2%)	1/26 (4%)
First incidence (days)	725 (T)	725 (T)	725 (T)	725 (T)
Life table test[d]	P=0.255N	P=0.349N	P=0.124N	P=0.288N
Logistic regression test[d]	P=0.255N	P=0.349N	P=0.124N	P=0.288N
Cochran-Armitage test[d]	P=0.182N			
Fisher exact test[d]		P=0.339N	P=0.181N	P=0.187N
Harderian Gland: Adenoma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	2.4%	8.6%	4.5%	6.7%
Terminal rate	0/36 (0%)	3/35 (9%)	2/44 (5%)	1/26 (4%)
First incidence (days)	700	725 (T)	725 (T)	407
Life table test	P=0.440	P=0.288	P=0.554	P=0.369
Logistic regression test	P=0.513	P=0.275	P=0.519	P=0.522
Cochran-Armitage test	P=0.586			
Fisher exact test		P=0.309	P=0.500	P=0.500
Liver: Hemangiosarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	4.9%	8.3%	2.3%	0.0%
Terminal rate	1/36 (3%)	2/35 (6%)	1/44 (2%)	0/26 (0%)
First incidence (days)	635	711	725 (T)	—[e]
Life table test	P=0.146N	P=0.477	P=0.449N	P=0.327N
Logistic regression test	P=0.151N	P=0.479	P=0.525N	P=0.292N
Cochran-Armitage test	P=0.104N			
Fisher exact test		P=0.500	P=0.500N	P=0.247N
Liver: Hepatocellular Adenoma				
Overall rate	21/50 (42%)	18/50 (36%)	12/50 (24%)	2/50 (4%)
Adjusted rate	52.1%	48.1%	26.6%	7.7%
Terminal rate	17/36 (47%)	16/35 (46%)	11/44 (25%)	2/26 (8%)
First incidence (days)	605	521	668	725 (T)
Life table test	P < 0.001N	P=0.396N	P=0.011N	P < 0.001N
Logistic regression test	P < 0.001N	P=0.447N	P=0.030N	P < 0.001N
Cochran-Armitage test	P < 0.001N			
Fisher exact test		P=0.341N	P=0.044N	P < 0.001N
Liver: Hepatocellular Carcinoma				
Overall rate	19/50 (38%)	14/50 (28%)	12/50 (24%)	2/50 (4%)
Adjusted rate	42.0%	32.2%	26.0%	7.2%
Terminal rate	11/36 (31%)	7/35 (20%)	10/44 (23%)	1/26 (4%)
First incidence (days)	485	464	556	636
Life table test	P=0.001N	P=0.279N	P=0.050N	P=0.002N
Logistic regression test	P=0.002N	P=0.129N	P=0.137N	P=0.001N
Cochran-Armitage test	P < 0.001N			
Fisher exact test		P=0.198N	P=0.097N	P < 0.001N

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

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	34/50 (68%)	27/50 (54%)	22/50 (44%)	4/50 (8%)
Adjusted rate	72.1%	62.2%	46.7%	14.6%
Terminal rate	23/36 (64%)	19/35 (54%)	19/44 (43%)	3/26 (12%)
First incidence (days)	485	464	556	636
Life table test	P < 0.001N	P=0.221N	P=0.004N	P < 0.001N
Logistic regression test	P < 0.001N	P=0.113N	P=0.016N	P < 0.001N
Cochran-Armitage test	P < 0.001N			
Fisher exact test		P=0.109N	P=0.013N	P < 0.001N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	8/50 (16%)	8/50 (16%)	10/50 (20%)	4/50 (8%)
Adjusted rate	22.2%	22.0%	22.7%	14.6%
Terminal rate	8/36 (22%)	7/35 (20%)	10/44 (23%)	3/26 (12%)
First incidence (days)	725 (T)	633	725 (T)	636
Life table test	P=0.300N	P=0.580	P=0.585	P=0.372N
Logistic regression test	P=0.366N	P=0.534	P=0.585	P=0.441N
Cochran-Armitage test	P=0.123N			
Fisher exact test		P=0.607N	P=0.398	P=0.178N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	6/50 (12%)	1/50 (2%)	5/50 (10%)	1/50 (2%)
Adjusted rate	15.9%	2.9%	11.0%	3.8%
Terminal rate	5/36 (14%)	1/35 (3%)	4/44 (9%)	1/26 (4%)
First incidence (days)	700	725 (T)	620	725 (T)
Life table test	P=0.221N	P=0.066N	P=0.383N	P=0.131N
Logistic regression test	P=0.246N	P=0.071N	P=0.477N	P=0.148N
Cochran-Armitage test	P=0.126N			
Fisher exact test		P=0.056N	P=0.500N	P=0.056N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	13/50 (26%)	9/50 (18%)	15/50 (30%)	5/50 (10%)
Adjusted rate	34.9%	24.8%	33.2%	18.3%
Terminal rate	12/36 (33%)	8/35 (23%)	14/44 (32%)	4/26 (15%)
First incidence (days)	700	633	620	636
Life table test	P=0.172N	P=0.262N	P=0.532N	P=0.139N
Logistic regression test	P=0.231N	P=0.306N	P=0.512	P=0.189N
Cochran-Armitage test	P=0.045N			
Fisher exact test		P=0.235N	P=0.412	P=0.033N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	1/50 (2%)	4/50 (8%)	0/50 (0%)	1/50 (2%)
Adjusted rate	2.8%	11.4%	0.0%	3.8%
Terminal rate	1/36 (3%)	4/35 (11%)	0/44 (0%)	1/26 (4%)
First incidence (days)	725 (T)	725 (T)	—	725 (T)
Life table test	P=0.431N	P=0.170	P=0.460N	P=0.688
Logistic regression test	P=0.431N	P=0.170	P=0.460N	P=0.688
Cochran-Armitage test	P=0.329N			
Fisher exact test		P=0.181	P=0.500N	P=0.753N

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

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.8%	11.4%	2.3%	3.8%
Terminal rate	1/36 (3%)	4/35 (11%)	1/44 (2%)	1/26 (4%)
First incidence (days)	725 (T)	725 (T)	725 (T)	725 (T)
Life table test	P=0.445N	P=0.170	P=0.716N	P=0.688
Logistic regression test	P=0.445N	P=0.170	P=0.716N	P=0.688
Cochran-Armitage test	P=0.331N			
Fisher exact test		P=0.181	P=0.753N	P=0.753N
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	5/50 (10%)	2/50 (4%)	0/50 (0%)
Adjusted rate	7.7%	13.0%	4.5%	0.0%
Terminal rate	2/36 (6%)	3/35 (9%)	2/44 (5%)	0/26 (0%)
First incidence (days)	635	511	725 (T)	—
Life table test	P=0.072N	P=0.339	P=0.424N	P=0.194N
Logistic regression test	P=0.058N	P=0.368	P=0.501N	P=0.182N
Cochran-Armitage test	P=0.041N			
Fisher exact test		P=0.357	P=0.500N	P=0.121N
All Organs: Malignant Lymphoma				
Overall rate	5/50 (10%)	3/50 (6%)	5/50 (10%)	1/50 (2%)
Adjusted rate	12.0%	7.9%	10.8%	2.8%
Terminal rate	3/36 (8%)	2/35 (6%)	3/44 (7%)	0/26 (0%)
First incidence (days)	438	562	668	382
Life table test	P=0.219N	P=0.378N	P=0.540N	P=0.212N
Logistic regression test	P=0.056N	P=0.245N	P=0.470	P=0.041N
Cochran-Armitage test	P=0.111N			
Fisher exact test		P=0.357N	P=0.630N	P=0.102N
All Organs: Benign Neoplasms				
Overall rate	29/50 (58%)	29/50 (58%)	22/50 (44%)	9/50 (18%)
Adjusted rate	70.4%	76.0%	48.8%	31.5%
Terminal rate	24/36 (67%)	26/35 (74%)	21/44 (48%)	7/26 (27%)
First incidence (days)	605	521	668	407
Life table test	P < 0.001N	P=0.494	P=0.017N	P=0.004N
Logistic regression test	P < 0.001N	P=0.380	P=0.058N	P=0.005N
Cochran-Armitage test	P < 0.001N			
Fisher exact test		P=0.580N	P=0.115N	P < 0.001N
All Organs: Malignant Neoplasms				
Overall rate	32/50 (64%)	24/50 (48%)	25/50 (50%)	4/50 (8%)
Adjusted rate	66.2%	50.5%	50.0%	13.4%
Terminal rate	20/36 (56%)	12/35 (34%)	19/44 (43%)	2/26 (8%)
First incidence (days)	438	421	556	382
Life table test	P < 0.001N	P=0.190N	P=0.047N	P < 0.001N
Logistic regression test	P < 0.001N	P=0.029N	P=0.262N	P < 0.001N
Cochran-Armitage test	P < 0.001N			
Fisher exact test		P=0.079N	P=0.113N	P < 0.001N

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

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	44/50 (88%)	42/50 (84%)	38/50 (76%)	13/50 (26%)
Adjusted rate	89.7%	87.4%	76.0%	42.6%
Terminal rate	31/36 (86%)	29/35 (83%)	32/44 (73%)	9/26 (35%)
First incidence (days)	438	421	556	382
Life table test	P < 0.001N	P=0.555N	P=0.019N	P < 0.001N
Logistic regression test	P < 0.001N	P=0.348N	P=0.164N	P < 0.001N
Cochran-Armitage test	P < 0.001N			
Fisher exact test		P=0.387N	P=0.096N	P < 0.001N

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

TABLE C4
Historical Incidence of Hepatocellular Neoplasms in Vehicle Control Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute			
Benzaldehyde	8/50	12/50	19/50
Furan	20/50	7/50	26/50
Furfural	9/50	7/50	16/50
p-Nitroaniline	19/50	10/50	25/50
Pentachloroanisole	20/50	9/50	26/50
Salicylazosulfapyridine	13/50	13/50	24/50
Overall Historical Incidence			
Total	267/813 (32.8%)	140/813 (17.2%)	364/813 (44.8%)
Standard deviation	13.1%	5.0%	14.1%
Range	14%-58%	8%-26%	25%-72%

[a] Data as of 12 May 1995

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

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	9	2	9
Natural deaths	5	6	4	15
Survivors				
Terminal sacrifice	36	35	44	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(49)	(49)	(48)	(43)
Inflammation, chronic		1 (2%)		
Intestine small, duodenum	(48)	(49)	(49)	(41)
Inflammation, chronic, focal	1 (2%)			
Necrosis, focal				1 (2%)
Intestine small, jejunum	(48)	(47)	(47)	(41)
Peyer's patch, hyperplasia, lymphoid			1 (2%)	
Intestine small, ileum	(48)	(48)	(47)	(38)
Peyer's patch, hyperplasia, lymphoid		1 (2%)	1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis		2 (4%)		
Basophilic focus		1 (2%)		1 (2%)
Clear cell focus	2 (4%)	2 (4%)	3 (6%)	
Congestion		1 (2%)		
Eosinophilic focus	6 (12%)	6 (12%)	9 (18%)	
Hepatodiaphragmatic nodule		1 (2%)		
Inflammation, chronic	24 (48%)	25 (50%)	16 (32%)	3 (6%)
Mineralization, focal				1 (2%)
Mixed cell focus		1 (2%)		
Necrosis, focal	5 (10%)	6 (12%)	3 (6%)	2 (4%)
Vacuolization cytoplasmic	10 (20%)	11 (22%)	6 (12%)	1 (2%)
Bile duct, cyst			1 (2%)	
Bile duct, hyperplasia	1 (2%)	2 (4%)	2 (4%)	
Hepatocyte, hypertrophy			1 (2%)	1 (2%)
Hepatocyte, karyomegaly	15 (30%)	14 (28%)	12 (24%)	2 (4%)
Mesentery	(10)	(4)	(2)	
Hemorrhage	2 (20%)			
Inflammation, chronic	1 (10%)	1 (25%)		
Thrombosis	1 (10%)			
Artery, inflammation, chronic	3 (30%)	1 (25%)		
Artery, thrombosis	1 (10%)			
Fat, necrosis	2 (20%)	1 (25%)	1 (50%)	
Pancreas	(50)	(49)	(49)	(50)
Inflammation, chronic		1 (2%)		
Acinus, atrophy, diffuse		2 (4%)		
Acinus, atrophy, focal	1 (2%)		1 (2%)	1 (2%)
Artery, hypertrophy	1 (2%)			
Duct, cyst	1 (2%)	2 (4%)		
Salivary glands	(50)	(50)	(50)	(49)
Vacuolization cytoplasmic				1 (2%)

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

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

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(49)
Inflammation, chronic	1 (2%)	3 (6%)	1 (2%)	
Ulcer	1 (2%)	3 (6%)		
Epithelium, hyperplasia	14 (28%)	12 (24%)	16 (32%)	13 (27%)
Stomach, glandular	(50)	(49)	(49)	(43)
Edema				1 (2%)
Erosion				1 (2%)
Hyperplasia			1 (2%)	
Mineralization	1 (2%)	4 (8%)	3 (6%)	1 (2%)
Glands, degeneration, cystic, focal	2 (4%)	7 (14%)	1 (2%)	2 (5%)
Tooth	(10)	(5)		
Developmental malformation	10 (100%)	5 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Inflammation, chronic, focal	1 (2%)	1 (2%)		
Mineralization				1 (2%)
Thrombosis	2 (4%)	1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Accessory adrenal cortical nodule	1 (2%)	1 (2%)		
Cyst	1 (2%)			
Cytoplasmic alteration, focal	1 (2%)	1 (2%)	2 (4%)	
Hyperplasia, focal		1 (2%)	2 (4%)	
Hypertrophy, focal	6 (12%)	7 (14%)	9 (18%)	7 (14%)
Capsule, hyperplasia, focal		2 (4%)		2 (4%)
Adrenal medulla	(49)	(50)	(49)	(49)
Hyperplasia	1 (2%)		1 (2%)	
Islets, pancreatic	(50)	(49)	(50)	(50)
Hyperplasia	4 (8%)	2 (4%)		
Parathyroid gland	(48)	(49)	(49)	(46)
Cyst	1 (2%)	1 (2%)	2 (4%)	
Pituitary gland	(49)	(50)	(48)	(44)
Angiectasis	1 (2%)	1 (2%)		
Pars distalis, cyst	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Pars distalis, cytoplasmic alteration, focal	2 (4%)			
Pars distalis, hyperplasia, focal	1 (2%)	3 (6%)	2 (4%)	2 (5%)
Thyroid gland	(50)	(50)	(50)	(50)
Degeneration, cystic, focal	6 (12%)	11 (22%)	10 (20%)	13 (26%)
Follicle, cyst	1 (2%)	6 (12%)	3 (6%)	2 (4%)
Follicular cell, hyperplasia	3 (6%)	1 (2%)	3 (6%)	1 (2%)
General Body System				
None				

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

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Genital System				
Epididymis	(49)	(50)	(50)	(50)
Granuloma sperm	1 (2%)	2 (4%)		
Inflammation, chronic	1 (2%)			
Spermatocele	1 (2%)			
Penis	(1)			
Angiectasis	1 (100%)			
Preputial gland	(49)	(48)	(50)	(50)
Degeneration, cystic	23 (47%)	31 (65%)	31 (62%)	12 (24%)
Inflammation, chronic	10 (20%)	8 (17%)	1 (2%)	3 (6%)
Inflammation, suppurative			1 (2%)	1 (2%)
Prostate	(50)	(49)	(50)	(50)
Inflammation, chronic		1 (2%)		1 (2%)
Epithelium, hyperplasia, focal	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Inflammation, chronic		2 (4%)		
Testes	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Mineralization, focal	1 (2%)			1 (2%)
Germinal epithelium, degeneration	1 (2%)			1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Angiectasis	1 (2%)			
Depletion cellular				1 (2%)
Hyperplasia	5 (10%)	2 (4%)		
Lymph node	(5)	(6)	(5)	(3)
Bronchial, hyperplasia, lymphoid				1 (33%)
Inguinal, hyperplasia, lymphoid	1 (20%)	2 (33%)		1 (33%)
Inguinal, pigmentation				1 (33%)
Mediastinal, hemorrhage	1 (20%)			
Pancreatic, hyperplasia, lymphoid		2 (33%)		
Renal, hyperplasia		1 (17%)		
Lymph node, mandibular	(42)	(47)	(47)	(47)
Hyperplasia, histiocytic	1 (2%)			
Hyperplasia, lymphoid			1 (2%)	2 (4%)
Necrosis				1 (2%)
Lymph node, mesenteric	(50)	(46)	(50)	(48)
Atrophy				2 (4%)
Ectasia	3 (6%)		1 (2%)	
Hematopoietic cell proliferation	1 (2%)			
Hemorrhage	17 (34%)	13 (28%)	9 (18%)	6 (13%)
Hyperplasia, lymphoid	1 (2%)		3 (6%)	2 (4%)
Pigmentation		1 (2%)		
Spleen	(50)	(49)	(50)	(49)
Depletion cellular	1 (2%)			12 (24%)
Fibrosis, focal	1 (2%)			
Hematopoietic cell proliferation	20 (40%)	27 (55%)	25 (50%)	13 (27%)
Hyperplasia, lymphoid			1 (2%)	
Thymus	(43)	(42)	(44)	(46)
Atrophy	2 (5%)	6 (14%)	4 (9%)	5 (11%)
Cyst	6 (14%)	6 (14%)	5 (11%)	3 (7%)
Necrosis		1 (2%)	1 (2%)	11 (24%)

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

	Vehicle Control	15 mg/kg	50 mg/kg	150 mg/kg
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Inflammation, chronic, focal				1 (2%)
Dermis, fibrosis		1 (2%)		
Epidermis, hyperplasia, focal			1 (2%)	
Subcutaneous tissue, edema	1 (2%)	2 (4%)	1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, hyperostosis		1 (2%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Necrosis	1 (2%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion		1 (2%)		4 (8%)
Foreign body		1 (2%)		
Hemorrhage	3 (6%)	1 (2%)		1 (2%)
Hyperplasia, histiocytic	4 (8%)		4 (8%)	1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	2 (4%)	1 (2%)	
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)			
Special Senses System				
Harderian gland	(1)	(4)	(2)	(2)
Inflammation, chronic, focal		1 (25%)		
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Congestion	1 (2%)			1 (2%)
Cyst	2 (4%)		1 (2%)	2 (4%)
Infarct			2 (4%)	2 (4%)
Inflammation, chronic	1 (2%)			
Inflammation, suppurative	1 (2%)	1 (2%)	1 (2%)	
Metaplasia, focal, osseous		1 (2%)	2 (4%)	1 (2%)
Nephropathy	46 (92%)	46 (94%)	45 (90%)	29 (58%)
Papilla, necrosis			1 (2%)	
Renal tubule, degeneration				7 (14%)
Renal tubule, dilatation				4 (8%)
Urethra	(3)	(3)	(1)	
Inflammation, chronic		1 (33%)		

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF THEOPHYLLINE

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	7	4	11	9
Natural deaths	6	9	5	8
Survivors				
Terminal sacrifice	37	37	34	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(46)	(48)	(47)	(49)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Intestine large, colon	(48)	(49)	(47)	(48)
Intestine large, cecum	(48)	(45)	(47)	(47)
Leiomyosarcoma		1 (2%)		1 (2%)
Intestine small, jejunum	(47)	(46)	(47)	(43)
Leiomyosarcoma, metastatic, uterus			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Hepatocellular carcinoma	8 (16%)	5 (10%)	6 (12%)	4 (8%)
Hepatocellular carcinoma, multiple	3 (6%)			1 (2%)
Hepatocellular adenoma	17 (34%)	9 (18%)	9 (18%)	3 (6%)
Hepatocellular adenoma, multiple	3 (6%)	2 (4%)	3 (6%)	
Hepatocholangiocarcinoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Histiocytic sarcoma	1 (2%)	6 (12%)	2 (4%)	2 (4%)
Ito cell tumor malignant, multiple		1 (2%)		
Mesentery	(10)	(8)	(6)	(5)
Hepatocholangiocarcinoma, metastatic, liver	1 (10%)	1 (13%)		1 (20%)
Histiocytic sarcoma		1 (13%)		1 (20%)
Sarcoma	1 (10%)			
Sarcoma, metastatic, skeletal muscle		1 (13%)		
Pancreas	(50)	(49)	(49)	(49)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	1 (2%)		1 (2%)
Histiocytic sarcoma				1 (2%)
Leiomyosarcoma, metastatic, uterus			1 (2%)	
Sarcoma, metastatic, skeletal muscle		1 (2%)		
Salivary glands	(50)	(49)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Stomach, forestomach	(50)	(49)	(49)	(50)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Squamous cell papilloma	4 (8%)	2 (4%)	2 (4%)	2 (4%)
Stomach, glandular	(49)	(49)	(48)	(50)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	1 (2%)		1 (2%)
Sarcoma, metastatic, skeletal muscle		1 (2%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(49)
Adenoma				1 (2%)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Capsule, adenoma	1 (2%)			
Capsule, hepatocholangiocarcinoma, metastatic, liver		1 (2%)		1 (2%)
Capsule, histiocytic sarcoma		2 (4%)		1 (2%)
Adrenal medulla	(50)	(48)	(50)	(49)
Pheochromocytoma benign	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Pituitary gland	(49)	(45)	(50)	(48)
Pars distalis, adenoma	9 (18%)	7 (16%)	7 (14%)	5 (10%)
Pars distalis, histiocytic sarcoma		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	4 (8%)	2 (4%)	3 (6%)	1 (2%)
General Body System				
None				
Genital System				
Ovary	(50)	(50)	(50)	(50)
Cystadenoma			1 (2%)	1 (2%)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	1 (2%)		1 (2%)
Histiocytic sarcoma		4 (8%)	1 (2%)	1 (2%)
Uterus	(50)	(50)	(50)	(50)
Histiocytic sarcoma		4 (8%)	3 (6%)	1 (2%)
Leiomyosarcoma			1 (2%)	
Endometrium, polyp stromal	1 (2%)		1 (2%)	1 (2%)
Endometrium, sarcoma stromal	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)
Histiocytic sarcoma		2 (4%)		1 (2%)
Lymph node	(6)	(8)	(7)	(6)
Histiocytic sarcoma		1 (13%)		
Iliac, histiocytic sarcoma		2 (25%)		
Inguinal, histiocytic sarcoma		1 (13%)		
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, liver		1 (13%)		
Mediastinal, hepatocholangiocarcinoma, metastatic, liver	1 (17%)	1 (13%)		
Mediastinal, histiocytic sarcoma		2 (25%)		
Renal, histiocytic sarcoma		2 (25%)		
Lymph node, mandibular	(48)	(49)	(44)	(43)
Histiocytic sarcoma		3 (6%)		
Lymph node, mesenteric	(48)	(46)	(48)	(45)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	1 (2%)		
Histiocytic sarcoma		3 (7%)		1 (2%)
Sarcoma, metastatic, mesentery	1 (2%)			
Sarcoma, metastatic, skeletal muscle		1 (2%)		

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Hematopoietic System (continued)				
Spleen	(50)	(50)	(49)	(48)
Hemangiosarcoma		2 (4%)		
Hepatocholangiocarcinoma, metastatic, liver				1 (2%)
Histiocytic sarcoma		2 (4%)		1 (2%)
Thymus	(47)	(44)	(49)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Histiocytic sarcoma		2 (5%)		1 (2%)
Integumentary System				
Skin	(49)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver				1 (2%)
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Subcutaneous tissue, hemangiosarcoma		1 (2%)		
Subcutaneous tissue, histiocytic sarcoma			1 (2%)	
Subcutaneous tissue, sarcoma	1 (2%)		1 (2%)	
Subcutaneous tissue, pinna, histiocytic sarcoma			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, fibrosarcoma			1 (2%)	
Skeletal muscle	(3)	(3)	(2)	(1)
Fibrosarcoma		1 (33%)		
Hepatocholangiocarcinoma, metastatic, liver	1 (33%)	1 (33%)		1 (100%)
Sarcoma	1 (33%)	1 (33%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Meninges, histiocytic sarcoma		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	4 (8%)	4 (8%)	3 (6%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)			
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)		
Hepatocellular carcinoma, metastatic, liver	3 (6%)			
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	1 (2%)		1 (2%)
Histiocytic sarcoma		4 (8%)	1 (2%)	1 (2%)
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Sarcoma, metastatic, skeletal muscle		1 (2%)		
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Mediastinum, hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Mediastinum, sarcoma, metastatic, uncertain primary site		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Special Senses System				
Harderian gland	(6)	(6)	(8)	(2)
Adenoma	6 (100%)	5 (83%)	4 (50%)	2 (100%)
Carcinoma			2 (25%)	
Histiocytic sarcoma		1 (17%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Histiocytic sarcoma		3 (6%)		
Urinary bladder	(49)	(49)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	8 (16%)	4 (8%)	2 (4%)
Lymphoma malignant	11 (22%)	8 (16%)	10 (20%)	8 (16%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	46	39	41	28
Total primary neoplasms	79	63	62	37
Total animals with benign neoplasms	33	27	27	18
Total benign neoplasms	49	33	36	20
Total animals with malignant neoplasms	26	24	22	17
Total malignant neoplasms	30	30	26	17
Total animals with metastatic neoplasms	5	4	1	1
Total metastatic neoplasms	15	20	2	9
Total animals with malignant neoplasms of uncertain primary site		1		

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

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: Vehicle Control

Number of Days on Study	5 0 1	5 5 5	5 6 1	5 8 3	6 0 5	6 2 4	6 2 7	6 4 2	6 4 3	6 4 3	6 5 4	6 5 5	6 8 4	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	2 1 0	2 2 5	2 4 8	2 1 8	2 2 8	2 1 1	2 4 0	2 0 5	2 0 7	2 4 3	2 5 0	2 2 6	2 4 7	2 0 1	2 0 2	2 0 3	2 0 4	2 0 6	2 0 8	2 0 9	2 1 2	2 1 3	2 1 4	2 1 5	2 1 6
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	M	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver					X																				
Intestine large, colon	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	A	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	A	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma	X			X					X																X
Hepatocellular carcinoma, multiple												X													
Hepatocellular adenoma						X		X		X				X		X	X			X			X		
Hepatocellular adenoma, multiple							X																		
Hepatocholangiocarcinoma					X																				
Histiocytic sarcoma																X									
Mesentery		+	+	+	+						+	+	+		+		+								
Hepatocholangiocarcinoma, metastatic, liver					X																				
Sarcoma													X												
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver					X																				
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver					X																				
Squamous cell papilloma																				X	X				
Stomach, glandular	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver					X																				
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver					X																				
Capsule, adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma										X							X		X			X	X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma															X										
General Body System																									
None																									

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

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	2 1 7	2 1 9	2 2 0	2 2 1	2 2 2	2 2 3	2 2 4	2 2 7	2 2 9	2 3 0	2 3 1	2 3 2	2 3 3	2 3 4	2 3 5	2 3 6	2 3 7	2 3 8	2 3 9	2 4 1	2 4 2	2 4 4	2 4 5	2 4 6	2 4 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Hepatocholangiocarcinoma, metastatic, liver																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma										X		X							X	X						8
Hepatocellular carcinoma, multiple								X									X									3
Hepatocellular adenoma		X	X		X								X					X	X	X	X			X		17
Hepatocellular adenoma, multiple						X					X															3
Hepatocholangiocarcinoma																										1
Histiocytic sarcoma																										1
Mesentery									+																	10
Hepatocholangiocarcinoma, metastatic, liver																										1
Sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocholangiocarcinoma, metastatic, liver																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocholangiocarcinoma, metastatic, liver																										1
Squamous cell papilloma		X													X											4
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocholangiocarcinoma, metastatic, liver																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocholangiocarcinoma, metastatic, liver																										1
Capsule, adenoma												X														1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign														X												1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma						X								X	X		X									9
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma													X				X							X		4
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	501	555	561	583	600	624	627	642	643	643	654	655	684	731	731	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	210	225	248	218	228	211	240	205	207	243	250	226	247	201	202	203	204	206	208	209	212	213	214	215	216
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver					X																				
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal																									
Endometrium, sarcoma stromal														X											
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mediastinal		+	+		+					+										+					
Hepatocholangiocarcinoma, metastatic, liver					X																				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver					X																				
Sarcoma, metastatic, mesentery													X												
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma												X													
Subcutaneous tissue, sarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle		+			+								+												
Hepatocholangiocarcinoma, metastatic, liver					X																				
Sarcoma													X												
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve							+	+																	
Spinal cord							+	+																	
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X																	
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma																									
Hepatocellular carcinoma, metastatic, liver				X																					X
Hepatocholangiocarcinoma, metastatic, liver					X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	2 1 7	2 1 9	2 2 0	2 2 1	2 2 2	2 2 3	2 2 4	2 2 7	2 2 9	2 3 0	2 3 1	2 3 2	2 3 3	2 3 4	2 3 5	2 3 6	2 3 7	2 3 8	2 3 9	2 4 1	2 4 2	2 4 4	2 4 5	2 4 6	2 4 9	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocholangiocarcinoma, metastatic, liver																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal																				X						1
Endometrium, sarcoma stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mediastinal																		+								6
Hepatocholangiocarcinoma, metastatic, liver																										1
Lymph node, mandibular	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	48
Hepatocholangiocarcinoma, metastatic, liver																										1
Sarcoma, metastatic, mesentery																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, sarcoma									X																	1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										3
Hepatocholangiocarcinoma, metastatic, liver																										1
Sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										2
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma					X																					2
Alveolar/bronchiolar adenoma, multiple																						X				1
Alveolar/bronchiolar carcinoma							X																			1
Hepatocellular carcinoma, metastatic, liver																	X									3
Hepatocholangiocarcinoma, metastatic, liver																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	501	555	561	583	605	624	627	642	643	643	654	655	684	731	731	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	210	225	248	118	228	111	140	005	007	043	500	226	447	001	012	033	044	066	088	099	112	133	144	155	166
Special Senses System																									
Eye																									
Harderian gland											+		+												+
Adenoma											X		X												X
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																X									
Lymphoma malignant		X	X							X										X					X

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: Vehicle Control (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	2 1 7	2 1 9	2 2 0	2 2 1	2 2 2	2 2 3	2 2 4	2 2 7	2 2 9	2 3 0	2 3 1	2 3 2	2 3 3	2 3 4	2 3 5	2 3 6	2 3 7	2 3 8	2 3 9	2 4 1	2 4 2	2 4 4	2 4 5	2 4 6	2 4 9	Total Tissues/ Tumors
Special Senses System																										
Eye																		+								1
Harderian gland																		+				+	+			6
Adenoma																		X				X	X			6
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant	X														X	X		X		X			X			11

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg

Number of Days on Study	4 9 4	5 8 4	5 9 1	6 0 3	6 0 4	6 1 1	6 3 3	6 3 7	6 4 4	6 5 2	6 5 8	6 8 7	7 0 2	7 2 5	7 2 5	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3
Carcass ID Number	2 7 9	3 0 0	2 8 3	2 7 8	2 6 6	2 9 4	2 9 7	2 5 8	2 9 1	2 6 9	2 6 0	2 6 5	2 9 3	2 7 6	2 8 9	2 5 1	2 5 2	2 5 3	2 5 4	2 5 5	2 5 6	2 5 7	2 5 9	2 6 1	2 6 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	A	+	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																									
Intestine small, duodenum	A	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	A	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	A	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma			X					X				X													
Hepatocellular adenoma																			X			X			X
Hepatocellular adenoma, multiple																							X		
Hepatocholangiocarcinoma										X															
Histiocytic sarcoma				X	X		X		X			X													
Ito cell tumor malignant, multiple																									
Mesentery	+									+								+							
Hepatocholangiocarcinoma, metastatic, liver										X															
Histiocytic sarcoma																									
Sarcoma, metastatic, skeletal muscle	X																								
Pancreas	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver										X															
Sarcoma, metastatic, skeletal muscle	X																								
Salivary glands	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X																					
Stomach, forestomach	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver										X															
Sarcoma, metastatic, skeletal muscle	X																								
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, hepatocholangiocarcinoma, metastatic, liver										X															
Capsule, histiocytic sarcoma				X					X																
Adrenal medulla	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign															X									X	
Islets, pancreatic	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+
Pituitary gland	I	+	+	+	I	I	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																			X						X
Pars distalis, histiocytic sarcoma				X																					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	2 6 3	2 6 4	2 6 7	2 6 8	2 7 0	2 7 1	2 7 2	2 7 3	2 7 4	2 7 5	2 7 7	2 8 0	2 8 1	2 8 2	2 8 4	2 8 5	2 8 6	2 8 7	2 8 8	2 9 0	2 9 2	2 9 5	2 9 6	2 9 8	2 9 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leiomyosarcoma												X														1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma													X					X								5
Hepatocellular adenoma		X										X		X	X			X				X				9
Hepatocellular adenoma, multiple													X													2
Hepatocholangiocarcinoma																										1
Histiocytic sarcoma												X														6
Ito cell tumor malignant, multiple											X															1
Mesentery							+		+			+	+					+								8
Hepatocholangiocarcinoma, metastatic, liver																										1
Histiocytic sarcoma												X														1
Sarcoma, metastatic, skeletal muscle																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocholangiocarcinoma, metastatic, liver																										1
Sarcoma, metastatic, skeletal muscle																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma				X											X											2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocholangiocarcinoma, metastatic, liver																										1
Sarcoma, metastatic, skeletal muscle																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, hepatocholangiocarcinoma, metastatic, liver																										1
Capsule, histiocytic sarcoma																										2
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma benign																										2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pars distalis, adenoma	X		X													X	X				X					7
Pars distalis, histiocytic sarcoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma					X											X										2

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	4 9 4	5 8 4	5 9 1	6 0 3	6 0 4	6 1 1	6 3 3	6 3 7	6 4 4	6 5 2	6 5 8	6 8 7	7 0 2	7 2 5	7 2 5	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3
Carcass ID Number	2 7 9	3 0 0	2 8 3	2 7 8	2 6 6	2 9 4	2 9 7	2 5 8	2 9 1	2 6 9	2 6 0	2 6 5	2 9 3	2 7 6	2 8 9	2 5 1	2 5 2	2 5 3	2 5 4	2 5 5	2 5 6	2 5 7	2 5 9	2 6 1	2 6 2
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver										X															
Histiocytic sarcoma				X	X		X		X																
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X	X				X																
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X	X																				
Lymph node		+		+	+		+			+			+						+				+		
Histiocytic sarcoma							X																		
Iliac, histiocytic sarcoma				X	X																				
Inguinal, histiocytic sarcoma				X																					
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, liver		X																							
Mediastinal, hepatocholangiocarcinoma, metastatic, liver										X															
Mediastinal, histiocytic sarcoma				X			X																		
Renal, histiocytic sarcoma					X																			X	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X	X		X																		
Lymph node, mesenteric	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver										X															
Histiocytic sarcoma				X	X		X																		
Sarcoma, metastatic, skeletal muscle	X																								
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma			X																						
Histiocytic sarcoma												X												X	
Thymus	+	+	+	M	+	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung		X																							
Histiocytic sarcoma					X		X																		
Integumentary System																									
Mammary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma			X																						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+					+				+															
Fibrosarcoma						X																			
Hepatocholangiocarcinoma, metastatic, liver										X															
Sarcoma	X																								

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	2 6 3	2 6 4	2 6 7	2 6 8	2 7 0	2 7 1	2 7 2	2 7 3	2 7 4	2 7 5	2 7 7	2 8 0	2 8 1	2 8 2	2 8 4	2 8 5	2 8 6	2 8 7	2 8 8	2 9 0	2 9 2	2 9 5	2 9 6	2 9 8	2 9 9	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocholangiocarcinoma, metastatic, liver																										1
Histiocytic sarcoma																										4
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma			X																							4
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymph node																										8
Histiocytic sarcoma																										1
Iliac, histiocytic sarcoma																										2
Inguinal, histiocytic sarcoma																										1
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, liver																										1
Mediastinal, hepatocholangiocarcinoma, metastatic, liver																										1
Mediastinal, histiocytic sarcoma																										2
Renal, histiocytic sarcoma																										2
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										3
Lymph node, mesenteric	+	+	+	I	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Hepatocholangiocarcinoma, metastatic, liver																										1
Histiocytic sarcoma																										3
Sarcoma, metastatic, skeletal muscle																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma									X																	2
Histiocytic sarcoma																										2
Thymus	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	44
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Histiocytic sarcoma																										2
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										3
Fibrosarcoma																										1
Hepatocholangiocarcinoma, metastatic, liver																										1
Sarcoma																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	4 9 4	5 8 4	5 9 1	6 0 3	6 0 4	6 1 1	6 3 3	6 3 7	6 4 4	6 5 2	6 5 8	6 8 7	7 0 2	7 2 5	7 2 5	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3
Carcass ID Number	2 7 9	3 0 0	2 8 3	2 7 8	2 6 6	2 9 4	2 9 7	2 5 8	2 9 1	2 6 9	2 6 0	2 6 5	2 9 3	2 7 6	2 8 9	2 5 1	2 5 2	2 5 3	2 5 4	2 5 5	2 5 6	2 5 7	2 5 9	2 6 1	2 6 2
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Meninges, histiocytic sarcoma				X																					
Peripheral nerve											I														
Spinal cord											+														
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X			X							X									
Alveolar/bronchiolar carcinoma		X																							
Hepatocholangiocarcinoma, metastatic, liver										X															
Histiocytic sarcoma				X	X		X					X													
Sarcoma, metastatic, uncertain primary site												X													
Sarcoma, metastatic, skeletal muscle	X																								
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung		X																							
Mediastinum, hepatocholangiocarcinoma, metastatic, liver										X															
Mediastinum, sarcoma, metastatic, uncertain primary site												X													
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X																					
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																			+						
Harderian gland				+															+						
Adenoma				X															X						
Histiocytic sarcoma				X																					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X					X															X	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma				X	X		X		X			X												X	
Lymphoma malignant													X	X					X						

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 7.5 mg/kg (continued)

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	2 6 3	2 6 4	2 6 7	2 6 8	2 7 0	2 7 1	2 7 2	2 7 3	2 7 4	2 7 5	2 7 7	2 8 0	2 8 1	2 8 2	2 8 4	2 8 5	2 8 6	2 8 7	2 8 8	2 9 0	2 9 2	2 9 5	2 9 6	2 9 8	2 9 9	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Meninges, histiocytic sarcoma																										1
Peripheral nerve																										
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																						X				4
Alveolar/bronchiolar carcinoma																										1
Hepatocholangiocarcinoma, metastatic, liver																										1
Histiocytic sarcoma																										4
Sarcoma, metastatic, uncertain primary site																										1
Sarcoma, metastatic, skeletal muscle																										1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Mediastinum, hepatocholangiocarcinoma, metastatic, liver																										1
Mediastinum, sarcoma, metastatic, uncertain primary site																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										1
Harderian gland						+		+																+	+	6
Adenoma								X																X	X	5
Histiocytic sarcoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma			X									X														8
Lymphoma malignant			X	X					X					X								X				8

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 25 mg/kg

Number of Days on Study	5 4 2	5 9 3	6 1 2	6 2 9	6 5 5	6 6 1	6 6 7	6 6 8	6 6 9	6 8 8	6 9 6	6 9 9	7 0 4	7 0 4	7 0 5	7 1 0	7 2 5	7 2 5	7 2 5	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2
Carcass ID Number	3 3 7	3 3 3	3 4 1	3 1 2	3 4 3	3 2 5	3 2 0	3 1 4	3 2 8	3 4 8	3 2 3	3 4 4	3 2 4	3 4 2	3 1 6	3 1 8	3 0 1	3 1 9	3 2 9	3 0 2	3 0 3	3 0 4	3 0 5	3 0 6	3 0 7
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Leiomyosarcoma, metastatic, uterus			X																						
Intestine small, ileum	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma							X			X		X												X	
Hepatocellular adenoma									X										X	X	X	X			
Hepatocellular adenoma, multiple																									
Hepatocholangiocarcinoma																									
Histiocytic sarcoma											X					X									
Mesentery												+	+									+	+		
Pancreas	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma, metastatic, uterus			X																						
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																	X								
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																				X	X		X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																				X					
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																									
Histiocytic sarcoma											X														
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma											X					X									
Leiomyosarcoma			X																						
Endometrium, polyp stromal																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 25 mg/kg (continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	3 0 8	3 0 9	3 1 0	3 1 1	3 1 3	3 1 5	3 1 7	3 2 1	3 2 2	3 2 6	3 2 7	3 3 0	3 3 1	3 3 2	3 3 4	3 3 5	3 3 6	3 3 8	3 3 9	3 4 0	3 4 5	3 4 6	3 4 7	3 4 9	3 5 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Leiomyosarcoma, metastatic, uterus																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma								X		X																6
Hepatocellular adenoma									X					X					X					X		9
Hepatocellular adenoma, multiple											X				X		X									3
Hepatocholangiocarcinoma				X																						1
Histiocytic sarcoma																										2
Mesentery				+								+														6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyosarcoma, metastatic, uterus																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma													X													2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign					X													X								2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	M	+	+	42
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma			X																		X		X		X	7
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																	X						X			3
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma																	X									1
Histiocytic sarcoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																			X							3
Leiomyosarcoma																										1
Endometrium, polyp stromal																				X						1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 25 mg/kg (continued)

Number of Days on Study	5 4 2	5 9 3	6 1 2	6 2 9	6 5 5	6 6 1	6 6 7	6 6 8	6 6 9	6 8 8	6 9 6	6 9 9	7 0 4	7 0 4	7 0 5	7 1 0	7 2 5	7 2 5	7 2 5	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2
Carcass ID Number	3 3 7	3 3 3	3 4 1	3 1 2	3 4 3	3 2 5	3 2 0	3 1 4	3 2 8	3 4 8	3 2 3	3 4 4	3 2 4	3 4 2	3 1 6	3 1 8	3 0 1	3 1 9	3 2 9	3 0 2	3 0 3	3 0 4	3 0 5	3 0 6	3 0 7
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+					+						+		+										
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	I	+	+	+	+	M	+	+	M	+	+	+	M	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, histiocytic sarcoma												X													
Subcutaneous tissue, sarcoma														X											
Subcutaneous tissue, pinna, histiocytic sarcoma												X													
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cranium, fibrosarcoma																									
Skeletal muscle									+										+						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve					+																				
Spinal cord					+																				
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X																				
Histiocytic sarcoma																X									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear									+																
Eye												+													
Harderian gland						+				+		+						+		+					
Adenoma										X		X						X							
Carcinoma						X														X					
Lacrimal gland																						+			
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma											X	X				X									
Lymphoma malignant		X					X	X				X	X						X						

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 25 mg/kg (continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	3 0 8	3 0 9	3 1 0	3 1 1	3 1 3	3 1 5	3 1 7	3 2 1	3 2 2	3 2 6	3 2 7	3 3 0	3 3 1	3 3 2	3 3 4	3 3 5	3 3 6	3 3 8	3 3 9	3 4 0	3 4 5	3 4 6	3 4 7	3 4 9	3 5 0	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node									+			+														7
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, histiocytic sarcoma																										1
Subcutaneous tissue, sarcoma																										1
Subcutaneous tissue, pinna, histiocytic sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cranium, fibrosarcoma			X																							1
Skeletal muscle																										2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma									X					X									X			4
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Eye																+										2
Harderian gland	+	+																					+			8
Adenoma	X																									4
Carcinoma																										2
Lacrimal gland																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																			X							4
Lymphoma malignant				X					X			X	X													10

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 75 mg/kg

Number of Days on Study	2 4 1	2 4 2	3 6 7	3 9 4	5 2 6	5 6 9	5 7 8	6 2 7	6 3 9	6 5 5	6 5 8	6 8 7	6 8 7	7 0 2	7 0 4	7 0 5	7 1 9	7 2 5	7 2 5	7 2 5	7 2 5	7 2 5	7 2 5	7 2 6
Carcass ID Number	3 6 2	3 7 4	3 8 5	3 8 0	3 9 9	3 8 9	3 5 3	3 5 7	3 6 5	3 5 9	3 7 5	3 7 6	3 8 4	3 7 1	3 8 2	3 9 1	3 9 5	3 5 8	3 6 0	3 6 6	3 7 8	3 8 3	3 8 6	4 0 0	3 7 7
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma												X													
Intestine small, duodenum	A	+	A	A	+	A	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	A
Intestine small, jejunum	A	+	A	A	+	A	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	A
Intestine small, ileum	A	+	A	A	+	A	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	A
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma															X					X					
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma																						X		X	
Hepatocholangiocarcinoma																	X								
Histiocytic sarcoma																X		X							
Mesentery				+			+										+	+						+	
Hepatocholangiocarcinoma, metastatic, liver																	X								
Histiocytic sarcoma																		X							
Pancreas	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver																	X								
Histiocytic sarcoma																		X							
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma								X																	
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver																	X								
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Capsule, hepatocholangiocarcinoma, metastatic, liver																	X								
Capsule, histiocytic sarcoma																		X							
Adrenal medulla	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Pars distalis, adenoma																									X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
None																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 75 mg/kg (continued)

Number of Days on Study	726	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	388	393	394	396	397	398	451	452	454	455	456	461	463	464	467	468	469	470	472	473	479	481	487	490	492	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Leiomyosarcoma																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma					X														X							4
Hepatocellular carcinoma, multiple			X																							1
Hepatocellular adenoma																		X								3
Hepatocholangiocarcinoma																										1
Histiocytic sarcoma																										2
Mesentery																										5
Hepatocholangiocarcinoma, metastatic, liver																										1
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocholangiocarcinoma, metastatic, liver																										1
Histiocytic sarcoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																				X						2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocholangiocarcinoma, metastatic, liver																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma			X																							1
Capsule, hepatocholangiocarcinoma, metastatic, liver																										1
Capsule, histiocytic sarcoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																	X									1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	M	+	+	M	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma		X				X									X		X									5
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																					X					1
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 75 mg/kg (continued)

Number of Days on Study	2 4 1	2 4 2	3 6 7	3 9 4	5 2 6	5 6 9	5 7 8	6 2 7	6 3 9	6 5 5	6 5 8	6 8 7	6 8 7	7 0 2	7 0 4	7 0 5	7 1 9	7 2 5	7 2 5	7 2 5	7 2 5	7 2 5	7 2 5	7 2 6
Carcass ID Number	3 6 2	3 7 4	3 8 5	3 8 0	3 9 9	3 8 9	3 5 3	3 5 7	3 6 5	3 5 9	3 7 5	3 7 6	3 8 4	3 7 1	3 8 2	3 9 1	3 9 5	3 5 8	3 6 0	3 6 6	3 7 8	3 8 3	3 8 6	4 0 0	3 7 7
Genital System																									
Clitoral gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma													X												
Hepatocholangiocarcinoma, metastatic, liver																	X								
Histiocytic sarcoma																		X							
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																X									
Endometrium, polyp stromal																X									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																		X							
Lymph node							+	+		+															
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	M	+	M	+	+	M	+	M	+
Lymph node, mesenteric	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+
Histiocytic sarcoma																		X							
Spleen	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver																	X								
Histiocytic sarcoma																		X							
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Histiocytic sarcoma																		X							
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver																	X								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																	+								
Hepatocholangiocarcinoma, metastatic, liver																	X								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve											+														
Spinal cord											+														
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Hepatocholangiocarcinoma, metastatic, liver																	X								
Histiocytic sarcoma																		X							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland																									
Adenoma																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 75 mg/kg (continued)

Number of Days on Study	726	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	38 8	39 3	39 4	39 6	39 7	39 8	51 1	52 2	54	55	56	61	63	64	67	68	69	70	72	73	79	81	87	90	92	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma																										1
Hepatocholangiocarcinoma, metastatic, liver																										1
Histiocytic sarcoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Endometrium, polyp stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node				+					+													+				6
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Lymph node, mesenteric	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocholangiocarcinoma, metastatic, liver																										1
Histiocytic sarcoma																										1
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocholangiocarcinoma, metastatic, liver																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Hepatocholangiocarcinoma, metastatic, liver																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X				X															X						3
Hepatocholangiocarcinoma, metastatic, liver																										1
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland											+								+							2
Adenoma											X								X							2

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 75 mg/kg (continued)

Number of Days on Study	2 4 1	2 4 2	3 6 7	3 9 4	5 2 6	5 6 9	5 7 8	6 2 7	6 3 9	6 5 5	6 5 8	6 8 7	6 8 7	7 0 2	7 0 4	7 0 5	7 1 9	7 2 5	7 2 5	7 2 5	7 2 5	7 2 5	7 2 5	7 2 5	7 2 6
Carcass ID Number	3 6 2	3 7 4	3 8 5	3 8 0	3 9 9	3 8 9	3 5 3	3 5 7	3 6 5	3 5 9	3 7 5	3 7 6	3 8 4	3 7 1	3 8 2	3 9 1	3 9 5	3 5 8	3 6 0	3 6 6	3 7 8	3 8 3	3 8 6	4 0 0	3 7 7
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																X		X							
Lymphoma malignant							X	X		X				X										X	

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Theophylline: 75 mg/kg (continued)

Number of Days on Study	7 2 6	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	3 8 8	3 9 3	3 9 4	3 9 6	3 9 7	3 9 8	3 5 1	3 5 2	3 5 4	3 5 5	3 5 6	3 6 1	3 6 3	3 6 4	3 6 7	3 6 8	3 6 9	3 7 0	3 7 2	3 7 3	3 7 9	3 8 1	3 8 7	3 9 0	3 9 2	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymphoma malignant				X					X								X									8

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Theophylline

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	6/50 (12%)	5/50 (10%)	4/50 (8%)	2/50 (4%)
Adjusted rate[b]	15.3%	12.7%	10.5%	6.1%
Terminal rate[c]	4/37 (11%)	4/37 (11%)	2/34 (6%)	2/33 (6%)
First incidence (days)	654	603	688	725 (T)
Life table test[d]	P=0.135N	P=0.497N	P=0.382N	P=0.164N
Logistic regression test[d]	P=0.117N	P=0.496N	P=0.093N	P=0.147N
Cochran-Armitage test[d]	P=0.105N			
Fisher exact test[d]		P=0.500N	P=0.370N	P=0.134N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	5/50 (10%)	6/50 (12%)	2/50 (4%)
Adjusted rate	15.3%	12.7%	15.3%	6.1%
Terminal rate	4/37 (11%)	4/37 (11%)	3/34 (9%)	2/33 (6%)
First incidence (days)	654	603	661	725 (T)
Life table test	P=0.143N	P=0.497N	P=0.614	P=0.164N
Logistic regression test	P=0.124N	P=0.496N	P=0.264N	P=0.147N
Cochran-Armitage test	P=0.111N			
Fisher exact test		P=0.500N	P=0.620N	P=0.134N
Liver: Hepatocellular Adenoma				
Overall rate	20/50 (40%)	11/50 (22%)	12/50 (24%)	3/50 (6%)
Adjusted rate	48.3%	29.7%	34.0%	9.1%
Terminal rate	16/37 (43%)	11/37 (30%)	11/34 (32%)	3/33 (9%)
First incidence (days)	624	725 (T)	669	725 (T)
Life table test	P < 0.001N	P=0.042N	P=0.098N	P < 0.001N
Logistic regression test	P < 0.001N	P=0.035N	P=0.049N	P < 0.001N
Cochran-Armitage test	P < 0.001N			
Fisher exact test		P=0.041N	P=0.066N	P < 0.001N
Liver: Hepatocellular Carcinoma				
Overall rate	11/50 (22%)	5/50 (10%)	6/50 (12%)	5/50 (10%)
Adjusted rate	26.0%	11.8%	15.3%	14.6%
Terminal rate	7/37 (19%)	2/37 (5%)	3/34 (9%)	4/33 (12%)
First incidence (days)	501	591	667	704
Life table test	P=0.217N	P=0.095N	P=0.164N	P=0.129N
Logistic regression test	P=0.156N	P=0.090N	P=0.171N	P=0.092N
Cochran-Armitage test	P=0.164N			
Fisher exact test		P=0.086N	P=0.143N	P=0.086N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	29/50 (58%)	14/50 (28%)	18/50 (36%)	8/50 (16%)
Adjusted rate	64.1%	34.5%	46.7%	23.4%
Terminal rate	21/37 (57%)	11/37 (30%)	14/34 (41%)	7/33 (21%)
First incidence (days)	501	591	667	704
Life table test	P=0.002N	P=0.005N	P=0.050N	P < 0.001N
Logistic regression test	P < 0.001N	P=0.002N	P=0.020N	P < 0.001N
Cochran-Armitage test	P < 0.001N			
Fisher exact test		P=0.002N	P=0.022N	P < 0.001N

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/50 (6%)	4/50 (8%)	4/50 (8%)	3/50 (6%)
Adjusted rate	7.6%	9.7%	10.8%	9.1%
Terminal rate	2/37 (5%)	2/37 (5%)	3/34 (9%)	3/33 (9%)
First incidence (days)	642	611	655	725 (T)
Life table test	P=0.567N	P=0.502	P=0.485	P=0.614
Logistic regression test	P=0.533N	P=0.494	P=0.505	P=0.645
Cochran-Armitage test	P=0.514N			
Fisher exact test		P=0.500	P=0.500	P=0.661N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	4/50 (8%)	3/50 (6%)
Adjusted rate	10.2%	11.6%	10.8%	9.1%
Terminal rate	3/37 (8%)	2/37 (5%)	3/34 (9%)	3/33 (9%)
First incidence (days)	642	584	655	725 (T)
Life table test	P=0.408N	P=0.506	P=0.623	P=0.557N
Logistic regression test	P=0.355N	P=0.489	P=0.634N	P=0.522N
Cochran-Armitage test	P=0.352N			
Fisher exact test		P=0.500	P=0.643N	P=0.500N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	9/49 (18%)	7/45 (16%)	7/50 (14%)	5/48 (10%)
Adjusted rate	24.1%	19.4%	20.6%	15.6%
Terminal rate	8/36 (22%)	7/36 (19%)	7/34 (21%)	5/32 (16%)
First incidence (days)	643	725 (T)	725 (T)	725 (T)
Life table test	P=0.261N	P=0.391N	P=0.432N	P=0.257N
Logistic regression test	P=0.209N	P=0.391N	P=0.340N	P=0.207N
Cochran-Armitage test	P=0.183N			
Fisher exact test		P=0.466N	P=0.376N	P=0.205N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	4/50 (8%)	2/50 (4%)	2/50 (4%)	2/50 (4%)
Adjusted rate	10.8%	5.4%	5.9%	5.3%
Terminal rate	4/37 (11%)	2/37 (5%)	2/34 (6%)	1/33 (3%)
First incidence (days)	725 (T)	725 (T)	725 (T)	627
Life table test	P=0.426N	P=0.336N	P=0.376N	P=0.384N
Logistic regression test	P=0.395N	P=0.336N	P=0.376N	P=0.356N
Cochran-Armitage test	P=0.375N			
Fisher exact test		P=0.339N	P=0.339N	P=0.339N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	4/50 (8%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	10.8%	5.4%	8.8%	3.0%
Terminal rate	4/37 (11%)	2/37 (5%)	3/34 (9%)	1/33 (3%)
First incidence (days)	725 (T)	725 (T)	725 (T)	725 (T)
Life table test	P=0.229N	P=0.336N	P=0.547N	P=0.214N
Logistic regression test	P=0.229N	P=0.336N	P=0.547N	P=0.214N
Cochran-Armitage test	P=0.191N			
Fisher exact test		P=0.339N	P=0.500N	P=0.181N

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	8/50 (16%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.7%	18.2%	10.4%	5.8%
Terminal rate	1/37 (3%)	3/37 (8%)	1/34 (3%)	1/33 (3%)
First incidence (days)	725 (T)	603	696	705
Life table test	P=0.305N	P=0.023	P=0.179	P=0.467
Logistic regression test	P=0.252N	P=0.016	P=0.189	P=0.488
Cochran-Armitage test	P=0.266N			
Fisher exact test		P=0.015	P=0.181	P=0.500
All Organs: Malignant Lymphoma				
Overall rate	11/50 (22%)	8/50 (16%)	10/50 (20%)	8/50 (16%)
Adjusted rate	26.6%	21.1%	24.3%	20.4%
Terminal rate	8/37 (22%)	7/37 (19%)	5/34 (15%)	4/33 (12%)
First incidence (days)	555	702	593	578
Life table test	P=0.445N	P=0.307N	P=0.526N	P=0.385N
Logistic regression test	P=368N	P=0.302N	P=0.541N	P=0.306N
Cochran-Armitage test	P=0.356N			
Fisher exact test		P=0.306N	P=0.500N	P=0.306N
All Organs: Benign Neoplasms				
Overall rate	33/50 (66%)	27/50 (54%)	27/50 (54%)	18/50 (36%)
Adjusted rate	76.6%	67.2%	70.6%	49.6%
Terminal rate	27/37 (74%)	24/37 (65%)	23/34 (68%)	15/33 (45%)
First incidence (days)	624	603	655	627
Life table test	P=0.013N	P=0.158N	P=0.252N	P=0.012N
Logistic regression test	P=0.004N	P=0.123N	P=0.079N	P=0.003N
Cochran-Armitage test	P=0.003N			
Fisher exact test		P=0.154N	P=0.154N	P=0.002N
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	24/50 (48%)	22/50 (44%)	17/50 (34%)
Adjusted rate	56.0%	48.7%	48.3%	41.1%
Terminal rate	17/37 (46%)	12/37 (32%)	11/34 (32%)	9/33 (27%)
First incidence (days)	501	494	593	578
Life table test	P=0.126N	P=0.424N	P=0.342N	P=0.140N
Logistic regression test	P=0.103N	P=0.445N	P=0.335N	P=0.059N
Cochran-Armitage test	P=0.039N			
Fisher exact test		P=0.421N	P=0.274N	P=0.053N

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	46/50 (92%)	39/50 (78%)	41/50 (82%)	28/50 (56%)
Adjusted rate	92.0%	79.5%	87.1%	66.6%
Terminal rate	33/37 (89%)	27/37 (73%)	28/34 (82%)	19/33 (58%)
First incidence (days)	501	494	593	578
Life table test	P=0.014N	P=0.154N	P=0.343N	P=0.010N
Logistic regression test	P < 0.001N	P=0.046N	P=0.118N	P < 0.001N
Cochran-Armitage test	P < 0.001N			
Fisher exact test		P=0.045N	P=0.117N	P < 0.001N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle 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 the vehicle controls and that dosed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by **N**.

TABLE D4
Historical Incidence of Hepatocellular Neoplasms in Vehicle Control Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute			
Benzaldehyde	1/50	1/50	2/50
Furan	5/50	2/50	7/50
Furfural	1/50	4/50	5/50
p-Nitroaniline	13/50	7/50	17/50
Pentachloroanisole	8/50	4/50	11/50
Salicylazosulfapyridine	12/50	2/50	14/50
Overall Historical Incidence			
Total	111/809 (13.7%)	40/809 (4.9%)	145/809 (17.9%)
Standard deviation	8.6%	3.6%	9.9%
Range	2%-28%	0%-14%	4%-37%

[a] Data as of 12 May 1995

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	7	4	11	9
Natural deaths	6	9	5	8
Survivors				
Terminal sacrifice	37	37	34	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Muscularis, inflammation, chronic	1 (2%)			
Gallbladder	(46)	(48)	(47)	(49)
Inflammation, chronic		1 (2%)		1 (2%)
Intestine large, colon	(48)	(49)	(47)	(48)
Inflammation, chronic				1 (2%)
Polyp inflammatory			1 (2%)	
Intestine large, cecum	(48)	(45)	(47)	(47)
Inflammation, chronic		1 (2%)		
Intestine small, jejunum	(47)	(46)	(47)	(43)
Peyer's patch, hyperplasia, lymphoid		2 (4%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)		1 (2%)	
Basophilic focus			1 (2%)	1 (2%)
Eosinophilic focus	6 (12%)	7 (14%)	5 (10%)	3 (6%)
Hematopoietic cell proliferation	2 (4%)	1 (2%)	1 (2%)	
Hyperplasia, focal, lymphoid		1 (2%)	2 (4%)	1 (2%)
Inflammation, chronic		3 (6%)	5 (10%)	2 (4%)
Inflammation, focal	1 (2%)			1 (2%)
Mixed cell focus	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Necrosis, focal	2 (4%)	4 (8%)	2 (4%)	1 (2%)
Pigmentation, focal		2 (4%)	1 (2%)	
Vacuolization cytoplasmic	6 (12%)	8 (16%)	5 (10%)	2 (4%)
Bile duct, cyst	1 (2%)			
Centrilobular, necrosis	1 (2%)	1 (2%)		1 (2%)
Hepatocyte, karyomegaly			1 (2%)	
Mesentery	(10)	(8)	(6)	(5)
Hemorrhage		1 (13%)		
Inflammation, chronic		1 (13%)		
Fat, necrosis	8 (80%)	4 (50%)	2 (33%)	2 (40%)
Pancreas	(50)	(49)	(49)	(49)
Inflammation, chronic		1 (2%)	1 (2%)	
Necrosis	1 (2%)			
Acinus, atrophy, focal			2 (4%)	
Duct, cyst	1 (2%)		3 (6%)	
Salivary glands	(50)	(49)	(50)	(50)
Fibrosis	1 (2%)		1 (2%)	1 (2%)
Mineralization				1 (2%)
Vacuolization cytoplasmic		1 (2%)	1 (2%)	

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

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Alimentary System (continued)				
Stomach, forestomach	(50)	(49)	(49)	(50)
Cyst		1 (2%)		
Edema	1 (2%)	1 (2%)		
Ulcer			2 (4%)	
Epithelium, hyperplasia	6 (12%)	6 (12%)	12 (24%)	5 (10%)
Stomach, glandular	(49)	(49)	(48)	(50)
Edema	1 (2%)			
Erosion	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic		1 (2%)		
Mineralization	4 (8%)	3 (6%)	5 (10%)	9 (18%)
Glands, degeneration, cystic, focal	11 (22%)	5 (10%)	4 (8%)	3 (6%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation, chronic				1 (2%)
Heart	(50)	(50)	(50)	(50)
Inflammation, chronic, focal	2 (4%)		1 (2%)	1 (2%)
Mineralization		1 (2%)		1 (2%)
Thrombosis				1 (2%)
Artery, inflammation, chronic		1 (2%)		
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(49)
Accessory adrenal cortical nodule	1 (2%)	1 (2%)		2 (4%)
Cyst	1 (2%)		1 (2%)	
Cytoplasmic alteration, focal		1 (2%)	3 (6%)	
Hematopoietic cell proliferation		1 (2%)	1 (2%)	
Hyperplasia, focal				1 (2%)
Hypertrophy, focal	1 (2%)	3 (6%)		1 (2%)
Adrenal medulla	(50)	(48)	(50)	(49)
Hyperplasia		1 (2%)	3 (6%)	
Islets, pancreatic	(50)	(49)	(49)	(49)
Hyperplasia		1 (2%)	1 (2%)	
Parathyroid gland	(49)	(47)	(42)	(45)
Cyst	1 (2%)	1 (2%)	2 (5%)	2 (4%)
Infiltration cellular, lymphocyte	1 (2%)			
Pituitary gland	(49)	(45)	(50)	(48)
Angiectasis	3 (6%)			1 (2%)
Hemorrhage				1 (2%)
Pars distalis, angiectasis	1 (2%)			
Pars distalis, cyst	1 (2%)	4 (9%)	1 (2%)	
Pars distalis, cytoplasmic alteration, focal	3 (6%)	1 (2%)	2 (4%)	2 (4%)
Pars distalis, hyperplasia, focal	4 (8%)	7 (16%)	14 (28%)	6 (13%)
Thyroid gland	(50)	(50)	(50)	(50)
Degeneration, cystic, focal	12 (24%)	13 (26%)	10 (20%)	15 (30%)
Inflammation, chronic, focal	1 (2%)			
Follicle, cyst	2 (4%)	11 (22%)	5 (10%)	7 (14%)
Follicular cell, hyperplasia	7 (14%)	8 (16%)	8 (16%)	11 (22%)
General Body System				
None				

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Genital System				
Clitoral gland	(50)	(49)	(49)	(49)
Degeneration, cystic	7 (14%)	3 (6%)	1 (2%)	6 (12%)
Inflammation, chronic	1 (2%)			1 (2%)
Ovary	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Cyst	6 (12%)	5 (10%)	5 (10%)	9 (18%)
Hemorrhage	1 (2%)	2 (4%)	5 (10%)	1 (2%)
Inflammation, suppurative				2 (4%)
Uterus	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Cyst	1 (2%)		2 (4%)	1 (2%)
Hemorrhage	2 (4%)	1 (2%)	1 (2%)	
Hydrometra	20 (40%)	19 (38%)	18 (36%)	13 (26%)
Inflammation, chronic			1 (2%)	
Inflammation, suppurative				1 (2%)
Endometrium, hyperplasia, cystic	48 (96%)	41 (82%)	43 (86%)	40 (80%)
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)
Depletion cellular				1 (2%)
Hyperplasia	4 (8%)	4 (8%)	4 (8%)	2 (4%)
Myelofibrosis		1 (2%)		
Necrosis			1 (2%)	
Lymph node	(6)	(8)	(7)	(6)
Hyperplasia			1 (14%)	
Iliac, inflammation, chronic			1 (14%)	
Inguinal, hyperplasia, lymphoid				1 (17%)
Mediastinal, hemorrhage	1 (17%)			
Lymph node, mandibular	(48)	(49)	(44)	(43)
Ectasia			1 (2%)	
Hemorrhage	2 (4%)	2 (4%)	1 (2%)	
Hyperplasia, lymphoid	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Lymph node, mesenteric	(48)	(46)	(48)	(45)
Atrophy				1 (2%)
Ectasia	1 (2%)			
Hemorrhage	2 (4%)	1 (2%)	2 (4%)	
Hyperplasia, lymphoid	1 (2%)		1 (2%)	1 (2%)
Inflammation, chronic			1 (2%)	
Spleen	(50)	(50)	(49)	(48)
Congestion	1 (2%)			
Depletion cellular				2 (4%)
Fibrosis, focal			1 (2%)	1 (2%)
Hematopoietic cell proliferation	13 (26%)	21 (42%)	19 (39%)	12 (25%)
Hyperplasia, lymphoid	5 (10%)	3 (6%)	2 (4%)	4 (8%)
Infarct		1 (2%)		
Necrosis, focal				1 (2%)
Thymus	(47)	(44)	(49)	(47)
Atrophy	2 (4%)	3 (7%)	2 (4%)	2 (4%)
Cyst	1 (2%)	1 (2%)		2 (4%)
Hemorrhage				1 (2%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)		
Necrosis	1 (2%)		1 (2%)	2 (4%)

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Integumentary System				
Mammary gland	(49)	(49)	(50)	(49)
Ectasia	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Hyperplasia	1 (2%)		3 (6%)	2 (4%)
Skin	(49)	(50)	(50)	(50)
Inflammation, chronic, focal	2 (4%)		2 (4%)	2 (4%)
Ulcer			2 (4%)	
Dermis, fibrosis		2 (4%)		3 (6%)
Epidermis, hyperplasia, focal			2 (4%)	1 (2%)
Pinna, ulcer			1 (2%)	
Subcutaneous tissue, edema		4 (8%)		1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy	9 (18%)	6 (12%)	11 (22%)	17 (34%)
Hyperostosis			1 (2%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Atrophy, focal	3 (6%)	1 (2%)	2 (4%)	2 (4%)
Hemorrhage	1 (2%)			1 (2%)
Necrosis			2 (4%)	
Spinal cord	(2)	(1)	(1)	(1)
Hemorrhage, focal	1 (50%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, histiocytic	2 (4%)			
Pigmentation	1 (2%)			
Thrombosis			1 (2%)	
Alveolar epithelium, hyperplasia		1 (2%)	2 (4%)	
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative			1 (2%)	
Mucosa, glands, dilatation, focal	2 (4%)	2 (4%)		1 (2%)
Nasolacrimal duct, cyst			1 (2%)	
Special Senses System				
Eye	(1)	(1)	(2)	
Atrophy	1 (100%)			
Cornea, inflammation, chronic		1 (100%)	2 (100%)	
Harderian gland	(6)	(6)	(8)	(2)
Hyperplasia, focal		1 (17%)		
Inflammation, chronic, focal			2 (25%)	

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

	Vehicle Control	7.5 mg/kg	25 mg/kg	75 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Congestion				1 (2%)
Hyperplasia, lymphoid		1 (2%)		
Infarct			1 (2%)	
Metaplasia, focal, osseous		2 (4%)	3 (6%)	
Nephropathy	25 (50%)	27 (54%)	27 (54%)	25 (50%)
Thrombosis			1 (2%)	
Pelvis, dilatation	1 (2%)			
Renal tubule, accumulation, hyaline droplet		3 (6%)		
Renal tubule, casts protein			1 (2%)	
Renal tubule, degeneration	3 (6%)		1 (2%)	4 (8%)
Renal tubule, dilatation		1 (2%)	1 (2%)	
Renal tubule, mineralization	1 (2%)			1 (2%)
Renal tubule, pigmentation			1 (2%)	1 (2%)
Urinary bladder	(49)	(49)	(49)	(50)
Hyperplasia, lymphoid	2 (4%)	2 (4%)		1 (2%)

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA MUTAGENICITY TEST PROTOCOL

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

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of theophylline. In the absence of toxicity, 10,000 μg/plate was selected as the high dose.

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

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al*. (1987). Theophylline was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and at least three doses of theophylline; the high dose was limited by toxicity. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9 and at theophylline concentrations that did not induce cell cycle delay, CHO cells were incubated for 26 hours with theophylline in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 24 hours prior to harvest. After 26 hours, the medium containing theophylline was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. Because significant chemical-induced cell cycle delay was seen at theophylline concentrations of 300 μg/mL and higher in cultures without S9, incubation time of these cultures was lengthened to ensure a sufficient number of scorable (second-division metaphase) cells. In the SCE test with S9, cells were incubated with theophylline, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no theophylline. Incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a

statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P < 0.005$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with theophylline for 20 hours; based on cell cycle information obtained in the SCE test, cell cycle delay was anticipated in cultures treated in the absence of S9, and the incubation period was extended beyond the standard 10 to 12 hours to permit accumulation of sufficient metaphase cells for analysis. After the 20-hour incubation, Colcemid was added and incubation continued for 2 more hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with theophylline and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. One hundred first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.*, 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

MOUSE BONE MARROW SISTER CHROMATID EXCHANGE TEST PROTOCOL

Testing was performed as reported by McFee (1991). A dose range-finding study was performed. The highest dose was limited by toxicity. Theophylline was tested for the induction of SCEs in mouse bone marrow using a single protocol. Male $B6C3F_1$ mice (five animals per dose group) were injected intraperitoneally with theophylline dissolved in corn oil (injection volume = 0.4 mL). Solvent control mice received equivalent injections of corn oil only. The positive control was dimethylbenzanthracene.

The mice were implanted subcutaneously with a BrdU tablet (McFee *et al.*, 1983) 24 hours before harvest (1 hour before treatment). The use of BrdU allowed selection of the appropriate cell population (cells in the second metaphase following treatment) for scoring. Two hours before sacrifice, the mice received an intraperitoneal injection of colchicine in saline. The animals were killed 23 hours after treatment (24 hours after BrdU dosing). One or both femurs were removed, and the marrow was flushed out with phosphate-buffered saline (pH 7.0). The cells were treated with a hypotonic salt solution, fixed, and dropped onto chilled slides. After a 24-hour drying period, the slides were stained using fluorescence-plus-Giemsa and scored.

Twenty-five second-division metaphase cells were scored from each of four animals per treatment. Responses were evaluated as SCEs/cell, and the data were analyzed by a trend test (Margolin *et al.*, 1986).

MOUSE BONE MARROW CHROMOSOMAL ABERRATIONS TEST PROTOCOL

Testing was performed as reported by McFee (1991). A dose range-finding study was performed. The high dose was limited by toxicity. Theophylline was tested for induction of Abs in mouse bone marrow in two different trials. The first trial used a standard harvest time of 17 hours and the second trial used a delayed harvest time of 36 hours.

Male B6C3F$_1$ mice (10 animals per dose group) were injected intraperitoneally with theophylline dissolved in corn oil (injection volume = 0.4 mL). Solvent control mice received equivalent injections of corn oil only. The positive control was dimethylbenzanthracene. The mice were subcutaneously implanted with a BrdU tablet (McFee *et al.*, 1983) 18 hours before the scheduled harvest (1 hour prior to theophylline injection for the standard protocol and 18 hours after theophylline injection for the delayed harvest protocol). The use of BrdU allowed selection of the appropriate cell population for scoring. (Abs induced by chemical administration are present in maximum number at the first metaphase following treatment; they decline in number during subsequent nuclear divisions due to cell death.) Two hours before sacrifice, the mice received an intraperitoneal injection of colchicine in saline. The animals were killed 17 or 36 hours after theophylline injection (18 hours after BrdU dosing). One or both femurs were removed and the marrow was flushed out with phosphate-buffered saline (pH 7.0). Cells were treated with a hypotonic salt solution, fixed, and dropped onto chilled slides. After a 24-hour drying period, the slides were stained and scored.

Fifty first-division metaphase cells were scored from each of eight animals per treatment. Responses were evaluated as the percentage of aberrant metaphase cells, excluding gaps. The data were analyzed by a trend test (Margolin *et al.*, 1986).

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay can be found in MacGregor *et al*. (1990). Peripheral blood samples were obtained from male and female B6C3F$_1$ mice at the end of the 14-week feed and gavage studies. Smears were immediately prepared and fixed in absolute methanol, stained with a chromatin-specific fluorescent dye mixture of Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983), and coded. Slides were scanned at 630× or 1,000× magnification using a semi-automated image analysis system to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes (NCEs) from each of 10 animals per dose group. The criteria of Schmid (1976) were used to define micronuclei, with the additional requirement that the micronuclei exhibit the characteristic fluorescent emissions of DNA (blue with 360 nm and orange with 540 nm UV illumination); the minimum size limit was approximately one-twentieth the diameter of the NCE.

The results were tabulated as the mean of the pooled results from all animals within a treatment group, plus or minus the standard error of the mean. The frequency of micronucleated cells among normochromatic erythrocytes was analyzed by a statistical software package that tested for increasing trend over exposure groups using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposure group and the control group (Margolin *et al.*, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dose group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 14-week feed and gavage studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitude of those effects.

RESULTS

Theophylline in concentrations from 100 to 10,000 μg/plate did not induce mutations in *Salmonella typhimurium* strain TA97, TA98, TA100, or TA1535 when included in the incubation medium with or without induced rat or hamster liver S9 (Table E1; Zeiger *et al.*, 1988). In cytogenetic tests with cultured CHO cells, theophylline induced SCEs in the absence of S9 activation at concentrations from 100 to 405 μg/mL (Table E2). Cell cycle delay was noted in cultures exposed to concentrations of 300 μg/mL or higher, and incubation time was lengthened accordingly. Theophylline did not induce Abs in cultured CHO cells, with or without S9 (Table E3).

Theophylline, administered to $B6C3F_1$ mice by intraperitoneal injection for a mouse bone marrow SCE assay, showed a significant, dose-related increase in SCEs at doses of 125 and 250 mg/kg (Table E4; McFee, 1991); however, a repeat trial was not performed, and therefore the response is unconfirmed. Theophylline, administered to $B6C3F_1$ mice by intraperitoneal injection, gave negative results in a mouse bone marrow Abs test that employed both standard (17 hours) and delayed harvest (36 hours) times (Table E5; McFee, 1991). Dose levels were limited by toxicity to 250 mg/kg in the standard harvest-time study and 150 mg/kg in the extended harvest-time study.

The frequency of micronucleated NCEs was measured in peripheral blood samples from male and female mice at the termination of the 14-week feed and gavage studies with theophylline (Tables E6 and E7). No significant increases in micronucleated erythrocytes were noted in peripheral blood of male or female mice.

In conclusion, theophylline showed limited evidence of mutagenicity. SCEs were observed after treatment of mammalian cells *in vitro* and *in vivo*, but negative results were seen in all other assays.

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

Strain	Dose (µg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA100	0	92 ± 0.9	150 ± 8.8	143 ± 11.8	167 ± 10.0	158 ± 6.4	172 ± 5.2
	100	101 ± 8.7	141 ± 11.3	149 ± 9.2	168 ± 5.0	144 ± 7.6	168 ± 14.2
	333	96 ± 12.5	137 ± 6.2	137 ± 2.8	180 ± 1.2	157 ± 7.0	150 ± 9.3
	1,000	93 ± 3.8	155 ± 8.0	138 ± 9.8	169 ± 5.3	148 ± 11.5	168 ± 9.2
	3,333	87 ± 10.9	138 ± 1.3	145 ± 12.7	149 ± 5.0	149 ± 7.8	144 ± 8.2
	10,000	66 ± 3.7	112 ± 19.1	125 ± 7.6	156 ± 16.6	133 ± 14.2	161 ± 7.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		437 ± 5.5	431 ± 12.4	1,245 ± 92.4	1,068 ± 37.2	614 ± 39.7	548 ± 19.5
TA1535	0	38 ± 2.0	44 ± 3.2	11 ± 2.7	16 ± 3.5	10 ± 1.5	11 ± 0.7
	100	33 ± 3.2	42 ± 3.2	8 ± 0.9	13 ± 2.0	9 ± 1.7	11 ± 1.2
	333	34 ± 4.3	37 ± 3.5	10 ± 1.7	11 ± 2.4	10 ± 2.1	12 ± 2.0
	1,000	29 ± 0.7	31 ± 3.3	9 ± 0.7	14 ± 2.0	9 ± 0.9	12 ± 3.2
	3,333	24 ± 3.2	33 ± 3.8	7 ± 0.7	10 ± 0.9	8 ± 1.8	10 ± 2.1
	10,000	13 ± 6.1	25 ± 4.4	6 ± 0.9	10 ± 2.1	5 ± 0.9	5 ± 0.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		435 ± 9.3	426 ± 36.3	498 ± 12.2	488 ± 85.5	215 ± 9.9	161 ± 9.2
TA97	0	147 ± 6.2	172 ± 12.9	142 ± 14.3	184 ± 12.0	191 ± 17.6	199 ± 9.2
	100	131 ± 2.3	209 ± 1.9	144 ± 3.8	212 ± 7.1	179 ± 0.9	257 ± 6.4
	333	127 ± 8.3	200 ± 12.2	139 ± 10.5	198 ± 6.1	168 ± 7.3	226 ± 4.2
	1,000	130 ± 11.2	171 ± 17.3	140 ± 17.6	201 ± 8.8	193 ± 9.0	231 ± 7.2
	3,333	125 ± 7.3	184 ± 3.0	131 ± 11.6	198 ± 1.7	172 ± 5.5	179 ± 11.4
	10,000	81 ± 4.2	132 ± 16.5	119 ± 9.3	185 ± 9.4	143 ± 11.5	170 ± 19.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,058 ± 81.4	1,622 ± 145.8	1,748 ± 53.5	1,723 ± 44.3	1,213 ± 19.9	720 ± 144.2
TA98	0	16 ± 3.5	19 ± 2.7	27 ± 1.2	32 ± 3.7	27 ± 2.6	39 ± 3.3
	100	20 ± 1.5	21 ± 4.8	26 ± 2.9	34 ± 1.5	26 ± 2.6	32 ± 4.1
	333	18 ± 1.3	20 ± 2.1	29 ± 1.2	44 ± 1.7	29 ± 5.0	35 ± 3.9
	1,000	12 ± 1.8	19 ± 3.8	33 ± 3.9	37 ± 4.3	27 ± 2.4	32 ± 4.9
	3,333	16 ± 2.3	16 ± 1.5	26 ± 3.9	28 ± 3.5	26 ± 5.5	21 ± 2.6
	10,000	9 ± 1.9	16 ± 2.0	21 ± 0.9	31 ± 0.6	18 ± 0.6	26 ± 3.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		619 ± 41.0	562 ± 16.2	959 ± 17.6	410 ± 27.5	333 ± 11.8	225 ± 22.5

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

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Theophylline[a]

Compound	Dose (µg/mL)	Total Cells Scored	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Trial 1								
Summary: Positive								
Dimethylsulfoxide		50	1,050	504	0.48	10.1	26.0	
Mitomycin-C	0.0015	50	1,046	776	0.74	15.5	26.0	54.56
	0.0100	5	105	223	2.12	44.6	26.0	342.46
Theophylline	10	50	1,050	533	0.50	10.7	26.0	5.75
	30	0[c]						
	100	50	1,049	668	0.63	13.4	26.0	32.66*
	300	50	1,033	713	0.69	14.3	34.0[d]	43.80*
	600	Toxic						
					P < 0.001[e]			
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,045	522	0.49	10.4	26.0	
Mitomycin-C	0.0015	50	1,042	712	0.68	14.2	26.0	36.79
	0.0100	5	103	241	2.33	48.2	26.0	368.41
Theophylline	201	50	1,049	828	0.78	16.6	26.0	58.02*
	300	50	1,041	886	0.85	17.7	32.0[d]	70.38*
	405	50	1,042	736	0.70	14.7	36.0[d]	41.40*
	510	Toxic						
					P < 0.001			
+S9								
Summary: Negative								
Dimethylsulfoxide		50	1,047	528	0.50	10.6	26.0	
Cyclophosphamide	0.4000	50	1,045	756	0.72	15.1	26.0	43.46
	2.0000	5	105	148	1.40	29.6	26.0	179.51
Theophylline	100	50	1,049	507	0.48	10.1	26.0	–4.16
	300	50	1,047	582	0.55	11.6	26.0	10.23
	600	50	1,048	536	0.51	10.7	26.0	1.42
					P=0.133			

* Positive response (≥20% increase over solvent control)
a The study was performed at Litton Bionetics, Inc. A detailed description of the protocol is presented in Galloway *et al*. (1987). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine
b SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells.
c The culture was contaminated with fungus and was not harvested.
d Because theophylline induced a delay in the cell division cycle at this concentration, harvest time was extended to maximize the proportion of second-division metaphase cells available for analysis.
e Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Theophylline[a]

-S9					+ S9				
Dose (µg/mL)	Total Cells Scored	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Trial 1 - Harvest time: 22.0 hours[b] Summary: Negative					**Trial 1** - Harvest time: 12.0 hours Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	14	0.14	11.0[c]		100	3	0.03	3.0
Mitomycin-C					Cyclophosphamide				
0.025	100	22	0.22	16.0	7.5	100	22	0.22	17.0
0.063	25	25	1.00	36.0	37.5	50	26	0.52	32.0
Theophylline					Theophylline				
510	100	7	0.07	6.0	510	100	2	0.02	2.0
555	100	8	0.08	6.0	555	100	2	0.02	2.0
600	100	9	0.09	8.0	600	100	5	0.05	4.0
				P=0.777[d]					P=0.345
Trial 2 - Harvest time: 22.0 hours[b] Summary: Negative									
Dimethylsulfoxide									
	100	8	0.08	5.0					
Mitomycin-C									
0.025	100	12	0.12	7.0					
0.063	50	24	0.48	36.0					
Theophylline									
510	100	4	0.04	4.0					
555	100	4	0.04	3.0					
600	100	2	0.02	2.0					
				P=0.888					

[a] The study was performed at Litton Bionetics, Inc. The detailed protocol is presented in Galloway *et al*. (1987).
Abs=aberrations
[b] Because of significant theophylline-induced cell cycle delay in the absence of S9, incubation time prior to the addition of Colcemid was lengthened to provide sufficient first-division metaphase cells at harvest.
[c] Unusually high background rate of aberrations; trial repeated to verify negative results
[d] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose

TABLE E4
Induction of Sister Chromatid Exchanges in Mouse Bone Marrow Cells by Theophylline[a]

Compound	Dose (mg/kg)	Mean SCEs/Cell
Corn Oil[b]		4.61 ± 0.48
Dimethylbenzanthracene[c]	2.5	9.38 ± 0.79
Theophylline	62.5	6.18 ± 0.43
	125.0	7.64 ± 0.84*
	250.0	8.46 ± 0.44*
		P=0.002[d]

* Significantly different from control ($P < 0.008$)
[a] The study was performed at Oak Ridge Associated Universities. The detailed protocol is presented in McFee (1991). Twenty-five second-division metaphase cells were scored from each of four animals per dose group. Data for mean SCEs/cells are given as mean ± standard error. SCE=sister chromatid exchange
[b] Solvent control; animals received corn oil by intraperitoneal injection.
[c] Positive control; dimethylbenzanthracene was dissolved in corn oil and administered by intraperitoneal injection.
[d] One-tailed trend analysis (Margolin *et al.*, 1986); significant at $P < 0.025$

TABLE E5
Induction of Chromosomal Aberrations in Mouse Bone Marrow Cells by Theophylline[a]

Compound	Dose (mg/kg)	Cells with Abs (%)
Trial 1 - Harvest time: 17 hours		
Corn Oil[b]		2.00
Dimethylbenzanthracene[c]	200	14.00
Theophylline	62.5	1.75
	125.0	2.50
	250.0	3.43
		P=0.304[d]
Trial 2 - Harvest time: 36 hours		
Corn Oil		2.25
Dimethylbenzanthracene	25.0	14.25
Theophylline	37.5	3.50
	75.0	2.00
	150.0	1.75
		P=0.774

[a] The study was performed at Oak Ridge Associated Universities. The detailed protocol and these data are presented in McFee (1991). Fifty first-division metaphase cells were scored from each of eight animals per dose group. Abs=aberrations
[b] Solvent control; animals received 0.4 mL corn oil by intraperitoneal injection.
[c] Positive control; dimethylbenzanthracene was dissolved in corn oil and administered by intraperitoneal injection.
[d] One-tailed trend analysis (Margolin *et al.*, 1986); significant at $P < 0.025$

TABLE E6
Frequency of Micronuclei in Normochromatic Peripheral Blood Erythrocytes from Mice Administered Theophylline in Feed for 14 Weeks[a]

Dose (ppm)	Micronucleated NCEs/1,000 NCEs[b]	Number of Mice
Male		
Control[c]	1.9 ± 0.2	10
Theophylline		
1,000	1.7 ± 0.1	10
2,000	2.0 ± 0.1	10
4,000	2.2 ± 0.2	10
	P=0.096[d]	
Female		
Control	1.3 ± 0.1	10
Theophylline		
1,000	1.7 ± 0.2	10
2,000	1.6 ± 0.1	10
4,000	1.8 ± 0.2	10
	P=0.034	

[a] The study was performed at USDA. Peripheral blood smears were prepared 24 hours after the final dosing. NCE=normochromatic erythrocyte
[b] Mean ± standard error
[c] Control animals received undosed feed.
[d] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at $P < 0.025$ (Margolin *et al.*, 1986)

TABLE E7
Frequency of Micronuclei in Normochromatic Peripheral Blood Erythrocytes from Mice Administered Theophylline by Gavage for 14 Weeks[a]

Dose (mg/kg)	Micronucleated NCEs/1,000 NCEs[b]	Number of Mice
Male		
Corn Oil[c]	1.9 ± 0.1	10
Theophylline		
75	1.8 ± 0.1	9
150	2.1 ± 0.2	10
300	2.4 ± 0.2	7
	P=0.123[d]	
Female		
Corn Oil	1.6 ± 0.2	9
Theophylline		
75	0.9 ± 0.1	10
150	1.5 ± 0.1	9
	P=0.621	

[a] The study was performed at USDA. Peripheral blood smears were prepared 24 hours after the final dosing. NCE=normochromatic erythrocyte
[b] Mean ± standard error
[c] Control animals received 0.4 mL corn oil by gavage.
[d] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at $P < 0.025$ (Margolin *et al.*, 1986)

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

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Feed Study of Theophylline[a]

	0 ppm	500 ppm	1,000 ppm	2,000 ppm	4,000 ppm	8,000 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	176 ± 5	192 ± 5	180 ± 4	176 ± 5	175 ± 3	132 ± 4**
Heart						
Absolute	0.702 ± 0.026	0.754 ± 0.051	0.658 ± 0.022	0.670 ± 0.011	0.640 ± 0.018	0.458 ± 0.007**
Relative	3.98 ± 0.07	3.93 ± 0.27	3.65 ± 0.06	3.81 ± 0.08	3.67 ± 0.10	3.48 ± 0.14*
R. Kidney						
Absolute	0.816 ± 0.052	0.894 ± 0.034	0.870 ± 0.021	0.896 ± 0.041	0.814 ± 0.020	0.612 ± 0.024**
Relative	4.62 ± 0.18	4.65 ± 0.11	4.83 ± 0.02	5.08 ± 0.14	4.67 ± 0.15	4.63 ± 0.18
Liver						
Absolute	7.508 ± 0.415	7.660 ± 0.149	7.096 ± 0.243	7.234 ± 0.297	6.944 ± 0.297	4.124 ± 0.169**
Relative	42.52 ± 1.49	39.93 ± 1.02	39.41 ± 0.88	41.01 ± 0.92	39.79 ± 1.63	31.15 ± 0.85**
Lung						
Absolute	1.012 ± 0.130	1.244 ± 0.129	1.090 ± 0.096	1.148 ± 0.089	0.986 ± 0.074	0.744 ± 0.031
Relative	5.70 ± 0.61	6.48 ± 0.68	6.10 ± 0.64	6.51 ± 0.46	5.66 ± 0.45	5.64 ± 0.29
R. Testis						
Absolute	0.841 ± 0.100	1.134 ± 0.040**	0.924 ± 0.065	1.058 ± 0.048	1.071 ± 0.030*	0.963 ± 0.026[b]
Relative	4.75 ± 0.51	5.90 ± 0.13	5.12 ± 0.30	5.99 ± 0.13**	6.15 ± 0.25**	7.17 ± 0.22**[b]
Thymus						
Absolute	0.428 ± 0.028	0.434 ± 0.033	0.438 ± 0.018	0.443 ± 0.032	0.403 ± 0.027	0.266 ± 0.011**
Relative	2.43 ± 0.16	2.25 ± 0.12	2.44 ± 0.13	2.52 ± 0.19	2.30 ± 0.12	2.02 ± 0.12
Female						
Necropsy body wt	130 ± 2	134 ± 3	138 ± 2	134 ± 4	130 ± 2	112 ± 2**
Heart						
Absolute	0.512 ± 0.017	0.500 ± 0.017	0.526 ± 0.029	0.496 ± 0.019	0.514 ± 0.015	0.426 ± 0.005*
Relative	3.95 ± 0.17	3.74 ± 0.10	3.81 ± 0.18	3.71 ± 0.10	3.97 ± 0.16	3.80 ± 0.06
R. Kidney						
Absolute	0.550 ± 0.023	0.558 ± 0.013	0.576 ± 0.016	0.536 ± 0.029	0.560 ± 0.010	0.490 ± 0.010
Relative	4.24 ± 0.18	4.18 ± 0.03	4.17 ± 0.08	4.00 ± 0.15	4.32 ± 0.08	4.37 ± 0.08
Liver						
Absolute	4.024 ± 0.121	4.344 ± 0.162	4.680 ± 0.089**	4.242 ± 0.172	4.214 ± 0.116	3.536 ± 0.032*
Relative	30.97 ± 0.43	32.53 ± 1.07	33.91 ± 0.48*	31.71 ± 0.64	32.53 ± 0.94	31.55 ± 0.68
Lung						
Absolute	0.708 ± 0.032	0.790 ± 0.020	0.774 ± 0.042	0.750 ± 0.041	0.766 ± 0.051	0.688 ± 0.028
Relative	5.46 ± 0.24	5.92 ± 0.10	5.61 ± 0.30	5.61 ± 0.24	5.90 ± 0.31	6.13 ± 0.23
R. Ovary						
Absolute	0.041 ± 0.004	0.040 ± 0.007	0.044 ± 0.005	0.036 ± 0.003	0.030 ± 0.004	0.026 ± 0.003
Relative	0.31 ± 0.03	0.30 ± 0.05	0.32 ± 0.04	0.27 ± 0.02	0.23 ± 0.03	0.24 ± 0.03
Thymus						
Absolute	0.330 ± 0.015	0.333 ± 0.020	0.372 ± 0.014	0.338 ± 0.022	0.330 ± 0.013	0.245 ± 0.017**
Relative	2.54 ± 0.08	2.49 ± 0.12	2.70 ± 0.11	2.52 ± 0.12	2.55 ± 0.11	2.18 ± 0.14
Uterus						
Absolute	0.248 ± 0.029	0.236 ± 0.032	0.250 ± 0.038	0.202 ± 0.044	0.200 ± 0.062	0.132 ± 0.026
Relative	1.91 ± 0.24	1.76 ± 0.23	1.81 ± 0.28	1.49 ± 0.31	1.56 ± 0.50	1.19 ± 0.25

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=4

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Gavage Study of Theophylline[a]: Comparison of Groups Receiving Once-Daily Administration

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Male						
n	5	5	5	5	5	0
Necropsy body wt	198 ± 2	201 ± 4	190 ± 6	178 ± 3**	166 ± 4**	—[b]
Brain						
Absolute	1.802 ± 0.009	1.828 ± 0.019	1.804 ± 0.031	1.750 ± 0.008	1.778 ± 0.005	—
Relative	9.10 ± 0.14	9.10 ± 0.20	9.53 ± 0.18	9.84 ± 0.11**	10.76 ± 0.23**	—
Heart						
Absolute	0.678 ± 0.020	0.676 ± 0.022	0.702 ± 0.027	0.634 ± 0.022	0.616 ± 0.017	—
Relative	3.42 ± 0.10	3.36 ± 0.07	3.71 ± 0.11	3.56 ± 0.08	3.73 ± 0.15	—
R. Kidney						
Absolute	0.742 ± 0.006	0.770 ± 0.011	0.736 ± 0.032	0.688 ± 0.018	0.656 ± 0.014**	—
Relative	3.75 ± 0.05	3.83 ± 0.08	3.88 ± 0.10	3.86 ± 0.05	3.97 ± 0.11	—
Liver						
Absolute	6.440 ± 0.098	6.292 ± 0.162	6.168 ± 0.262	5.678 ± 0.204**	5.178 ± 0.021**	—
Relative	32.49 ± 0.28	31.27 ± 0.43	32.50 ± 0.65	31.87 ± 0.82	31.33 ± 0.65	—
Lung						
Absolute	0.888 ± 0.013	0.918 ± 0.052	0.902 ± 0.044	0.864 ± 0.024	0.882 ± 0.070	—
Relative	4.48 ± 0.07	4.55 ± 0.20	4.77 ± 0.25	4.85 ± 0.12	5.31 ± 0.36*	—
R. Testis						
Absolute	1.197 ± 0.017	1.139 ± 0.093	1.193 ± 0.022	1.098 ± 0.019[c]	1.108 ± 0.038	—
Relative	6.04 ± 0.09	5.68 ± 0.48	6.30 ± 0.15	6.11 ± 0.10[c]	6.69 ± 0.09	—
Thymus						
Absolute	0.429 ± 0.020	0.451 ± 0.037	0.462 ± 0.036	0.389 ± 0.032	0.306 ± 0.012*	—
Relative	2.16 ± 0.08	2.23 ± 0.15	2.42 ± 0.13	2.16 ± 0.16	1.85 ± 0.10	—

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Gavage Study of Theophylline: Comparison of Groups Receiving Once-Daily Administration (continued)

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Female						
n	5	5	5	5	5	0
Necropsy body wt	129 ± 4	131 ± 3	128 ± 1	126 ± 2	122 ± 2	—
Brain						
Absolute	1.704 ± 0.016	1.680 ± 0.017	1.678 ± 0.023	1.656 ± 0.012	1.654 ± 0.018	—
Relative	13.23 ± 0.38	12.82 ± 0.25	13.09 ± 0.21	13.16 ± 0.20	13.52 ± 0.14	—
Heart						
Absolute	0.486 ± 0.022	0.466 ± 0.015	0.460 ± 0.010	0.474 ± 0.005	0.488 ± 0.017	—
Relative	3.77 ± 0.18	3.56 ± 0.13	3.59 ± 0.08	3.77 ± 0.09	3.98 ± 0.11	—
R. Kidney						
Absolute	0.534 ± 0.024	0.504 ± 0.012	0.494 ± 0.013	0.502 ± 0.012	0.512 ± 0.022	—
Relative	4.13 ± 0.10	3.85 ± 0.11	3.85 ± 0.10	3.98 ± 0.07	4.18 ± 0.14	—
Liver						
Absolute	4.166 ± 0.111	4.260 ± 0.125	4.000 ± 0.070	4.076 ± 0.099	4.220 ± 0.094	—
Relative	32.27 ± 0.59	32.46 ± 0.60	31.20 ± 0.44	32.36 ± 0.68	34.47 ± 0.51*	—
Lung						
Absolute	0.708 ± 0.024	0.718 ± 0.027	0.682 ± 0.016	0.696 ± 0.015	0.700 ± 0.023	—
Relative	5.49 ± 0.20	5.49 ± 0.27	5.32 ± 0.14	5.54 ± 0.20	5.71 ± 0.13	—
R. Ovary						
Absolute	0.044 ± 0.008	0.035 ± 0.003	0.046 ± 0.004	0.031 ± 0.002	0.034 ± 0.002	—
Relative	0.34 ± 0.06	0.27 ± 0.03	0.36 ± 0.03	0.25 ± 0.02	0.28 ± 0.02	—
Thymus						
Absolute	0.364 ± 0.023	0.341 ± 0.014	0.335 ± 0.014	0.293 ± 0.026*	0.244 ± 0.015**	—
Relative	2.81 ± 0.14	2.60 ± 0.07	2.62 ± 0.12	2.32 ± 0.17*	1.99 ± 0.10**	—
Uterus						
Absolute	0.342 ± 0.055	0.224 ± 0.042	0.254 ± 0.054	0.128 ± 0.006**	0.156 ± 0.014*	—
Relative	2.62 ± 0.37	1.73 ± 0.35	1.98 ± 0.42	1.01 ± 0.07**	1.27 ± 0.11*	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] All animals died before the end of the study.
[c] n=4

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Gavage Study of Theophylline[a]: Comparisons of Once-Daily to Twice-Daily Administration

	Low-dose Comparison		Mid-dose Comparison		High-dose Comparison[b]	
	12.5 mg/kg twice daily	25 mg/kg once daily	50 mg/kg twice daily	100 mg/kg once daily	200 mg/kg twice daily	400 mg/kg once daily
Male						
n	5	5	5	5	0	0
Necropsy body wt	188 ± 5	201 ± 4	182 ± 4	178 ± 3	—	—
Brain						
Absolute	1.754 ± 0.022	1.828 ± 0.019*	1.760 ± 0.022	1.750 ± 0.008	—	—
Relative	9.34 ± 0.28	9.10 ± 0.20	9.70 ± 0.21	9.84 ± 0.11	—	—
Heart						
Absolute	0.666 ± 0.030	0.676 ± 0.022	0.638 ± 0.019	0.634 ± 0.022	—	—
Relative	3.53 ± 0.11	3.36 ± 0.07	3.51 ± 0.11	3.56 ± 0.08	—	—
R. Kidney						
Absolute	0.700 ± 0.034	0.770 ± 0.011	0.710 ± 0.025	0.688 ± 0.018	—	—
Relative	3.71 ± 0.08	3.83 ± 0.08	3.91 ± 0.11	3.86 ± 0.05	—	—
Liver						
Absolute	6.092 ± 0.227	6.292 ± 0.162	5.886 ± 0.147	5.678 ± 0.204	—	—
Relative	32.30 ± 0.42	31.27 ± 0.43	32.38 ± 0.25	31.87 ± 0.82	—	—
Lung						
Absolute	0.904 ± 0.040	0.918 ± 0.052	0.866 ± 0.024	0.864 ± 0.024	—	—
Relative	4.80 ± 0.14	4.55 ± 0.20	4.77 ± 0.12	4.85 ± 0.12	—	—
R. Testis						
Absolute	1.126 ± 0.057	1.139 ± 0.093	1.092 ± 0.053	1.098 ± 0.019[c]	—	—
Relative	5.96 ± 0.18	5.68 ± 0.48	5.99 ± 0.19	6.11 ± 0.10[c]	—	—
Thymus						
Absolute	0.449 ± 0.015	0.451 ± 0.037	0.397 ± 0.012	0.389 ± 0.032[c]	—	—
Relative	2.39 ± 0.11	2.23 ± 0.15	2.19 ± 0.07	2.16 ± 0.16[c]	—	—

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Gavage Study of Theophylline: Comparisons of Once-Daily to Twice-Daily Administration (continued)

	Low-dose Comparison		Mid-dose Comparison		High-dose Comparison	
	12.5 mg/kg twice daily	25 mg/kg once daily	50 mg/kg twice daily	100 mg/kg once daily	200 mg/kg twice daily	400 mg/kg once daily
Female						
n	5	5	5	5	1	0
Necropsy body wt	123 ± 2	131 ± 3*	130 ± 2	126 ± 2	—	—
Brain						
Absolute	1.662 ± 0.030	1.680 ± 0.017	1.674 ± 0.007	1.656 ± 0.012	—	—
Relative	13.47 ± 0.25	12.82 ± 0.25	12.89 ± 0.17	13.16 ± 0.20	—	—
Heart						
Absolute	0.483 ± 0.022[c]	0.466 ± 0.015	0.474 ± 0.016	0.474 ± 0.005	—	—
Relative	3.95 ± 0.15[c]	3.56 ± 0.13	3.65 ± 0.17	3.77 ± 0.09	—	—
R. Kidney						
Absolute	0.472 ± 0.015	0.504 ± 0.012	0.500 ± 0.015	0.502 ± 0.012	—	—
Relative	3.82 ± 0.11	3.85 ± 0.11	3.85 ± 0.10	3.98 ± 0.07	—	—
Liver						
Absolute	3.832 ± 0.197	4.260 ± 0.125	4.116 ± 0.170	4.076 ± 0.099	—	—
Relative	31.00 ± 1.21	32.46 ± 0.60	31.64 ± 1.05	32.36 ± 0.68	—	—
Lung						
Absolute	0.688 ± 0.017	0.718 ± 0.027	0.716 ± 0.014	0.696 ± 0.015	—	—
Relative	5.57 ± 0.11	5.49 ± 0.27	5.51 ± 0.08	5.54 ± 0.20	—	—
R. Ovary						
Absolute	0.039 ± 0.007	0.035 ± 0.003	0.044 ± 0.006	0.031 ± 0.002	—	—
Relative	0.32 ± 0.06	0.27 ± 0.03	0.34 ± 0.04	0.25 ± 0.02	—	—
Thymus						
Absolute	0.315 ± 0.021	0.341 ± 0.014	0.326 ± 0.013	0.293 ± 0.026	—	—
Relative	2.55 ± 0.14	2.60 ± 0.07	2.51 ± 0.07	2.32 ± 0.17	—	—
Uterus						
Absolute	0.244 ± 0.033	0.224 ± 0.042	0.230 ± 0.019[c]	0.128 ± 0.006**[c]	—	—
Relative	1.99 ± 0.28	1.73 ± 0.35	1.75 ± 0.13[c]	1.01 ± 0.07**[c]	—	—

* Significantly different ($P \leq 0.05$) from the twice-daily administration group by a *t*-test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] All animals in the 400 mg/kg once daily and all but one female in the 200 mg/kg twice-daily groups died before the end of the study. These high-dose comparisons were not done.
[c] n=4

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Feed Study of Theophylline[a]

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Male				
n	10	10	10	10
Necropsy body wt	351 ± 8	368 ± 6	364 ± 4	344 ± 6
Brain				
Absolute	1.957 ± 0.040	1.986 ± 0.020	2.011 ± 0.016	1.933 ± 0.016
Relative	5.58 ± 0.08	5.41 ± 0.07	5.54 ± 0.07	5.63 ± 0.07
Heart				
Absolute	0.983 ± 0.026	1.007 ± 0.031	0.993 ± 0.020	0.953 ± 0.028
Relative	2.80 ± 0.04	2.74 ± 0.06	2.73 ± 0.04	2.77 ± 0.04
R. Kidney				
Absolute	1.283 ± 0.031	1.395 ± 0.037*	1.397 ± 0.028*	1.446 ± 0.047**
Relative	3.67 ± 0.10	3.79 ± 0.06	3.84 ± 0.06	4.19 ± 0.07**
Liver				
Absolute	12.899 ± 0.377	13.735 ± 0.437	13.581 ± 0.333	13.265 ± 0.353
Relative	36.80 ± 0.91	37.30 ± 0.68	37.33 ± 0.67	38.60 ± 0.99
Lung				
Absolute	1.470 ± 0.052	1.512 ± 0.056	1.549 ± 0.059	1.575 ± 0.062
Relative	4.20 ± 0.14	4.12 ± 0.16	4.25 ± 0.12	4.57 ± 0.14
R. Testis				
Absolute	1.440 ± 0.046	1.484 ± 0.025	1.491 ± 0.018	1.441 ± 0.029
Relative	4.10 ± 0.05	4.04 ± 0.07	4.11 ± 0.06	4.19 ± 0.05
Thymus				
Absolute	0.318 ± 0.019	0.296 ± 0.016	0.300 ± 0.012	0.283 ± 0.014
Relative	0.90 ± 0.04	0.80 ± 0.04	0.82 ± 0.03	0.82 ± 0.03

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Feed Study of Theophylline (continued)

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Female				
n	10	10	10	10
Necropsy body wt	207 ± 3	222 ± 3	206 ± 5	202 ± 8
Brain				
Absolute	1.843 ± 0.012[b]	1.878 ± 0.012	1.853 ± 0.015	1.820 ± 0.016
Relative	8.91 ± 0.14[b]	8.48 ± 0.13	9.02 ± 0.21	9.18 ± 0.44
Heart				
Absolute	0.664 ± 0.013[b]	0.675 ± 0.011	0.668 ± 0.015	0.649 ± 0.027
Relative	3.21 ± 0.05[b]	3.04 ± 0.05	3.25 ± 0.08	3.22 ± 0.06
R. Kidney				
Absolute	0.759 ± 0.017	0.808 ± 0.016	0.774 ± 0.025	0.790 ± 0.034
Relative	3.67 ± 0.07	3.65 ± 0.10	3.76 ± 0.10	3.92 ± 0.04
Liver				
Absolute	6.848 ± 0.240	7.233 ± 0.206	6.905 ± 0.187	7.062 ± 0.416
Relative	33.04 ± 0.78	32.65 ± 0.99	33.49 ± 0.60	34.81 ± 1.23
Lung				
Absolute	0.985 ± 0.015	1.047 ± 0.017	1.016 ± 0.031	1.100 ± 0.043*
Relative	4.76 ± 0.06	4.72 ± 0.07	4.93 ± 0.10	5.54 ± 0.32**
R. Ovary				
Absolute	0.055 ± 0.006	0.053 ± 0.002	0.064 ± 0.009	0.046 ± 0.003
Relative	0.27 ± 0.03	0.24 ± 0.01	0.31 ± 0.04	0.23 ± 0.02
Thymus				
Absolute	0.243 ± 0.011	0.249 ± 0.008	0.232 ± 0.015	0.200 ± 0.022
Relative	1.17 ± 0.04	1.12 ± 0.03	1.12 ± 0.05	0.97 ± 0.09
Uterus				
Absolute	0.509 ± 0.037	0.589 ± 0.067	0.584 ± 0.045	0.449 ± 0.061
Relative	2.47 ± 0.19	2.66 ± 0.30	2.83 ± 0.20	2.20 ± 0.27

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

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Gavage Study of Theophylline[a]

	Vehicle Control	37.5 mg/kg	75 mg/kg	150 mg/kg
Male				
n	10	10	10	9
Necropsy body wt	336 ± 5	334 ± 5	329 ± 5	321 ± 5
Brain				
Absolute	2.068 ± 0.072	2.012 ± 0.018	1.990 ± 0.011	1.989 ± 0.012
Relative	6.16 ± 0.20	6.02 ± 0.07	6.07 ± 0.09	6.22 ± 0.09
Heart				
Absolute	1.003 ± 0.021	0.944 ± 0.022	0.946 ± 0.011	0.953 ± 0.022
Relative	2.99 ± 0.08	2.82 ± 0.05	2.88 ± 0.04	2.98 ± 0.05
R. Kidney				
Absolute	1.251 ± 0.019	1.251 ± 0.036	1.246 ± 0.018	1.283 ± 0.029
Relative	3.73 ± 0.06	3.74 ± 0.08	3.79 ± 0.03	4.01 ± 0.08*
Liver				
Absolute	12.864 ± 0.322	12.235 ± 0.243	12.212 ± 0.353	12.179 ± 0.435
Relative	38.30 ± 0.62	36.59 ± 0.56	37.14 ± 0.80	37.92 ± 0.90
Lung				
Absolute	1.389 ± 0.026	1.380 ± 0.056	1.407 ± 0.048	1.367 ± 0.073
Relative	4.15 ± 0.11	4.12 ± 0.15	4.29 ± 0.15	4.26 ± 0.19
R. Testis				
Absolute	1.471 ± 0.020	1.553 ± 0.039	1.496 ± 0.011	1.429 ± 0.027
Relative	4.39 ± 0.05	4.64 ± 0.06*	4.56 ± 0.08	4.46 ± 0.08
Thymus				
Absolute	0.263 ± 0.008	0.240 ± 0.008	0.233 ± 0.012[b]	0.207 ± 0.013**
Relative	0.78 ± 0.02	0.72 ± 0.02	0.70 ± 0.04[b]	0.65 ± 0.04**

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Gavage Study of Theophylline
(continued)

	Vehicle Control	37.5 mg/kg	75 mg/kg	150 mg/kg
Female				
n	10	10	10	9
Necropsy body wt	203 ± 2	198 ± 2	209 ± 3	216 ± 3**
Brain				
Absolute	1.865 ± 0.023	1.882 ± 0.019	1.874 ± 0.026	1.859 ± 0.015
Relative	9.21 ± 0.13	9.49 ± 0.12	8.95 ± 0.05	8.62 ± 0.10**
Heart				
Absolute	0.673 ± 0.014	0.696 ± 0.022	0.710 ± 0.026	0.738 ± 0.026
Relative	3.32 ± 0.06	3.50 ± 0.09	3.39 ± 0.10	3.42 ± 0.11
R. Kidney				
Absolute	0.762 ± 0.017	0.803 ± 0.025	0.814 ± 0.028	0.839 ± 0.021
Relative	3.76 ± 0.08	4.04 ± 0.10	3.89 ± 0.11	3.88 ± 0.08
Liver				
Absolute	6.914 ± 0.125	6.839 ± 0.208	7.661 ± 0.221*	8.152 ± 0.253**
Relative	34.12 ± 0.52	34.50 ± 1.10	36.56 ± 0.75	37.78 ± 1.21*
Lung				
Absolute	1.043 ± 0.031[b]	1.082 ± 0.014	1.120 ± 0.038	1.114 ± 0.047
Relative	5.11 ± 0.14[b]	5.46 ± 0.08	5.35 ± 0.16	5.15 ± 0.18
R. Ovary				
Absolute	0.046 ± 0.004	0.062 ± 0.006	0.057 ± 0.004	0.049 ± 0.006
Relative	0.23 ± 0.02	0.31 ± 0.03*	0.27 ± 0.02	0.23 ± 0.03
Thymus				
Absolute	0.233 ± 0.010	0.221 ± 0.008	0.232 ± 0.006	0.194 ± 0.005**
Relative	1.15 ± 0.04	1.12 ± 0.04	1.11 ± 0.03	0.90 ± 0.03**
Uterus				
Absolute	0.595 ± 0.072	0.525 ± 0.044	0.587 ± 0.067	0.416 ± 0.034
Relative	2.95 ± 0.38	2.64 ± 0.20	2.82 ± 0.34	1.92 ± 0.16*

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

TABLE F6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Feed Study of Theophylline[a]

	0 ppm	500 ppm	1,000 ppm	2,000 ppm	4,000 ppm	8,000 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	22.8 ± 0.5	24.0 ± 0.5	23.6 ± 0.5	23.2 ± 0.7	23.0 ± 0.5	22.6 ± 0.7
Heart						
Absolute	0.112 ± 0.006	0.120 ± 0.003	0.114 ± 0.006	0.118 ± 0.007	0.108 ± 0.009	0.094 ± 0.007
Relative	4.91 ± 0.19	5.01 ± 0.16	4.82 ± 0.19	5.08 ± 0.27	4.68 ± 0.31	4.15 ± 0.20
R. Kidney						
Absolute	0.198 ± 0.006	0.206 ± 0.004	0.206 ± 0.012	0.208 ± 0.008	0.196 ± 0.006	0.170 ± 0.005*
Relative	8.68 ± 0.14	8.59 ± 0.19	8.73 ± 0.46	8.96 ± 0.18	8.52 ± 0.21	7.53 ± 0.19*
Liver						
Absolute	1.262 ± 0.053	1.208 ± 0.032	1.134 ± 0.032	1.206 ± 0.048	1.100 ± 0.043	1.128 ± 0.046
Relative	55.27 ± 1.50	50.37 ± 1.38*	48.06 ± 0.95**	51.92 ± 0.84	47.77 ± 1.11**	49.87 ± 0.78*
Lung						
Absolute	0.160 ± 0.010	0.164 ± 0.014	0.152 ± 0.008	0.162 ± 0.007	0.160 ± 0.014	0.140 ± 0.008
Relative	7.02 ± 0.43	6.85 ± 0.62	6.46 ± 0.41	7.03 ± 0.47	6.97 ± 0.64	6.17 ± 0.19
R. Testis						
Absolute	0.096 ± 0.003	0.097 ± 0.003	0.104 ± 0.002	0.101 ± 0.003	0.094 ± 0.005	0.091 ± 0.003
Relative	4.21 ± 0.08	4.06 ± 0.13	4.41 ± 0.13	4.37 ± 0.23	4.10 ± 0.26	4.05 ± 0.23
Thymus						
Absolute	0.045 ± 0.005	0.045 ± 0.006	0.042 ± 0.004	0.039 ± 0.004	0.055 ± 0.006	0.049 ± 0.004
Relative	1.97 ± 0.26	1.87 ± 0.25	1.78 ± 0.16	1.68 ± 0.20	2.40 ± 0.28	2.19 ± 0.21
Female						
Necropsy body wt	17.8 ± 0.7	18.0 ± 0.3	17.0 ± 0.6	18.2 ± 0.4	18.6 ± 0.8	19.4 ± 0.5
Heart						
Absolute	0.090 ± 0.004	0.094 ± 0.005	0.084 ± 0.002	0.092 ± 0.006	0.080 ± 0.006	0.092 ± 0.005
Relative	5.06 ± 0.19	5.22 ± 0.23	4.97 ± 0.27	5.05 ± 0.30	4.28 ± 0.22	4.75 ± 0.27
R. Kidney						
Absolute	0.136 ± 0.010	0.150 ± 0.007	0.134 ± 0.006	0.138 ± 0.008	0.138 ± 0.006	0.150 ± 0.010
Relative	7.62 ± 0.39	8.34 ± 0.39	7.92 ± 0.46	7.57 ± 0.35	7.42 ± 0.16	7.76 ± 0.60
Liver						
Absolute	0.930 ± 0.049	0.844 ± 0.018	0.838 ± 0.031	0.842 ± 0.023	0.888 ± 0.061	0.952 ± 0.047
Relative	52.17 ± 1.31	46.91 ± 0.92*	49.28 ± 0.67	46.29 ± 1.10*	47.54 ± 1.55	49.04 ± 1.73
Lung						
Absolute	0.144 ± 0.011	0.136 ± 0.005	0.164 ± 0.040	0.134 ± 0.010	0.142 ± 0.015	0.150 ± 0.014
Relative	8.06 ± 0.40	7.56 ± 0.27	9.57 ± 2.12	7.38 ± 0.61	7.61 ± 0.70	7.75 ± 0.74
R. Ovary						
Absolute	0.011 ± 0.004	0.010 ± 0.002	0.010 ± 0.001	0.010 ± 0.002	0.006 ± 0.001	0.006 ± 0.001
Relative	0.61 ± 0.19	0.54 ± 0.08	0.57 ± 0.05	0.54 ± 0.10	0.33 ± 0.07	0.30 ± 0.07
Thymus						
Absolute	0.066 ± 0.002	0.061 ± 0.001	0.054 ± 0.006	0.056 ± 0.002	0.055 ± 0.006	0.069 ± 0.001
Relative	3.72 ± 0.11	3.42 ± 0.12	3.16 ± 0.31	3.10 ± 0.09	2.94 ± 0.27*	3.58 ± 0.15
Uterus						
Absolute	0.112 ± 0.026	0.108 ± 0.018	0.120 ± 0.026	0.126 ± 0.024	0.056 ± 0.012	0.130 ± 0.020
Relative	6.19 ± 1.29	6.04 ± 1.07	6.98 ± 1.36	6.89 ± 1.24	2.95 ± 0.59	6.69 ± 1.01

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

TABLE F7
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Gavage Study of Theophylline[a]: Comparison of Groups Receiving Once-Daily Administration

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Male						
n	5	5	5	5	5	2
Necropsy body wt	25.4 ± 0.5	25.2 ± 0.7	25.0 ± 0.6	24.8 ± 0.4	26.2 ± 0.7	26.0 ± 0.0
Brain						
Absolute	0.460 ± 0.009	0.450 ± 0.008	0.454 ± 0.004	0.456 ± 0.006	0.478 ± 0.015	0.460 ± 0.000
Relative	18.15 ± 0.61	17.92 ± 0.65	18.21 ± 0.54	18.39 ± 0.17	18.32 ± 0.88	17.69 ± 0.00
Heart						
Absolute	0.130 ± 0.004	0.118 ± 0.005	0.120 ± 0.005	0.140 ± 0.008	0.128 ± 0.007	0.115 ± 0.005
Relative	5.13 ± 0.21	4.69 ± 0.17	4.80 ± 0.17	5.63 ± 0.28	4.89 ± 0.22	4.42 ± 0.19
R. Kidney						
Absolute	0.232 ± 0.007	0.226 ± 0.007	0.228 ± 0.006	0.228 ± 0.014	0.244 ± 0.010	0.215 ± 0.015
Relative	9.16 ± 0.39	8.98 ± 0.25	9.14 ± 0.30	9.18 ± 0.46	9.33 ± 0.39	8.27 ± 0.58
Liver						
Absolute	1.232 ± 0.039	1.144 ± 0.036	1.176 ± 0.036	1.148 ± 0.050	1.174 ± 0.029	1.120 ± 0.040
Relative	48.52 ± 1.35	45.39 ± 0.46	47.07 ± 1.27	46.24 ± 1.53	44.85 ± 0.67	43.08 ± 1.54
Lung						
Absolute	0.168 ± 0.012	0.150 ± 0.006	0.150 ± 0.009	0.160 ± 0.006	0.172 ± 0.012	0.150 ± 0.000
Relative	6.64 ± 0.55	5.96 ± 0.24	6.01 ± 0.39	6.46 ± 0.30	6.59 ± 0.50	5.77 ± 0.00
R. Testis						
Absolute	0.100 ± 0.004	0.096 ± 0.004	0.100 ± 0.003	0.102 ± 0.002	0.108 ± 0.004	0.103 ± 0.002
Relative	3.95 ± 0.13	3.84 ± 0.18	3.99 ± 0.10	4.13 ± 0.10	4.14 ± 0.16	3.96 ± 0.08
Thymus						
Absolute	0.046 ± 0.006	0.042 ± 0.005	0.049 ± 0.003	0.048 ± 0.004	0.045 ± 0.004	0.039 ± 0.001
Relative	1.83 ± 0.25	1.67 ± 0.21	1.97 ± 0.12	1.93 ± 0.15	1.72 ± 0.14	1.50 ± 0.04

TABLE F7
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Gavage Study of Theophylline: Comparison of Groups Receiving Once-Daily Administration (continued)

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Female						
n	5	5	5	5	5	0
Necropsy body wt	21.0 ± 0.3	21.6 ± 0.4	20.4 ± 0.4	20.0 ± 0.6	22.2 ± 0.4	—[b]
Brain						
Absolute	0.460 ± 0.005	0.456 ± 0.007	0.458 ± 0.004	0.438 ± 0.011	0.460 ± 0.003	—
Relative	21.91 ± 0.16	21.15 ± 0.60	22.48 ± 0.40	21.94 ± 0.58	20.74 ± 0.24	—
Heart						
Absolute	0.103 ± 0.005[c]	0.108 ± 0.004	0.108 ± 0.005	0.110 ± 0.006	0.110 ± 0.003	—
Relative	4.88 ± 0.16[c]	5.01 ± 0.23	5.31 ± 0.30	5.53 ± 0.40	4.96 ± 0.20	—
R. Kidney						
Absolute	0.158 ± 0.005	0.160 ± 0.006	0.166 ± 0.002	0.152 ± 0.006	0.158 ± 0.007	—
Relative	7.52 ± 0.18	7.42 ± 0.35	8.15 ± 0.16	7.62 ± 0.36	7.10 ± 0.22	—
Liver						
Absolute	1.072 ± 0.021	1.048 ± 0.041	1.064 ± 0.019	0.930 ± 0.027*	1.018 ± 0.036	—
Relative	51.10 ± 1.30	48.65 ± 2.40	52.31 ± 2.00	46.53 ± 0.90	45.84 ± 1.28*	—
Lung						
Absolute	0.146 ± 0.007	0.144 ± 0.009	0.144 ± 0.010	0.152 ± 0.007	0.146 ± 0.007	—
Relative	6.96 ± 0.36	6.68 ± 0.44	7.07 ± 0.54	7.61 ± 0.34	6.59 ± 0.34	—
R. Ovary						
Absolute	0.010 ± 0.002	0.012 ± 0.002	0.012 ± 0.001	0.012 ± 0.002	0.012 ± 0.001	—
Relative	0.49 ± 0.08	0.54 ± 0.08	0.57 ± 0.04	0.62 ± 0.10	0.55 ± 0.07	—
Thymus						
Absolute	0.067 ± 0.005	0.066 ± 0.002	0.065 ± 0.003	0.055 ± 0.003	0.065 ± 0.004	—
Relative	3.18 ± 0.23	3.05 ± 0.09	3.17 ± 0.18	2.77 ± 0.13	2.91 ± 0.18	—
Uterus						
Absolute	0.142 ± 0.010	0.117 ± 0.016	0.139 ± 0.019	0.172 ± 0.010	0.139 ± 0.013	—
Relative	6.77 ± 0.41	5.48 ± 0.80	6.74 ± 0.81	8.61 ± 0.48	6.25 ± 0.57	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] All animals died before the end of the study.
[c] n=4

TABLE F8
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Gavage Study of Theophylline[a]: Comparisons of Once-Daily to Twice-Daily Administration

	Low-dose Comparison		Mid-dose Comparison		High-dose Comparison	
	12.5 mg/kg twice daily	25 mg/kg once daily	50 mg/kg twice daily	100 mg/kg once daily	200 mg/kg twice daily	400 mg/kg once daily
Male						
n	5	5	5	5	5	2
Necropsy body wt	24.8 ± 0.5	25.2 ± 0.7	24.8 ± 0.5	24.8 ± 0.4	25.6 ± 0.5	26.0 ± 0.0
Brain						
Absolute	0.454 ± 0.007	0.450 ± 0.008	0.462 ± 0.010	0.456 ± 0.006	0.466 ± 0.007	0.460 ± 0.000
Relative	18.32 ± 0.21	17.92 ± 0.65	18.64 ± 0.31	18.39 ± 0.17	18.23 ± 0.37	17.69 ± 0.00
Heart						
Absolute	0.116 ± 0.007	0.118 ± 0.005	0.122 ± 0.004	0.140 ± 0.008	0.130 ± 0.006	0.115 ± 0.005
Relative	4.66 ± 0.23	4.69 ± 0.17	4.92 ± 0.08	5.63 ± 0.28*	5.09 ± 0.29	4.42 ± 0.19
R. Kidney						
Absolute	0.204 ± 0.015	0.226 ± 0.007	0.222 ± 0.007	0.228 ± 0.014	0.220 ± 0.008	0.215 ± 0.015
Relative	8.20 ± 0.51	8.98 ± 0.25	8.95 ± 0.11	9.18 ± 0.46	8.59 ± 0.28	8.27 ± 0.58
Liver						
Absolute	1.148 ± 0.034	1.144 ± 0.036	1.082 ± 0.030	1.148 ± 0.050	1.084 ± 0.047	1.120 ± 0.040
Relative	46.30 ± 1.05	45.39 ± 0.46	43.60 ± 0.49	46.24 ± 1.53	42.28 ± 1.13	43.08 ± 1.54
Lung						
Absolute	0.152 ± 0.011	0.150 ± 0.006	0.168 ± 0.009	0.160 ± 0.006	0.168 ± 0.011	0.150 ± 0.000
Relative	6.11 ± 0.37	5.96 ± 0.24	6.76 ± 0.26	6.46 ± 0.30	6.57 ± 0.47	5.77 ± 0.00
R. Testis						
Absolute	0.105 ± 0.003	0.096 ± 0.004	0.096 ± 0.002	0.102 ± 0.002	0.103 ± 0.003	0.103 ± 0.002
Relative	4.24 ± 0.04	3.84 ± 0.18	3.89 ± 0.08	4.13 ± 0.10	4.03 ± 0.13	3.96 ± 0.08
Thymus						
Absolute	0.047 ± 0.005	0.042 ± 0.005	0.042 ± 0.002	0.048 ± 0.004	0.041 ± 0.004	0.039 ± 0.001
Relative	1.88 ± 0.17	1.67 ± 0.21	1.68 ± 0.11	1.93 ± 0.15	1.62 ± 0.15	1.50 ± 0.04

TABLE F8
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Gavage Study of Theophylline: Comparisons of Once-Daily to Twice-Daily Administration (continued)

	Low-dose Comparison		Mid-dose Comparison		High-dose Comparison[b]	
	12.5 mg/kg twice daily	25 mg/kg once daily	50 mg/kg twice daily	100 mg/kg once daily	200 mg/kg twice daily	400 mg/kg once daily
Female						
n	5	5	5	5	5	0
Necropsy body wt	21.6 ± 0.7	21.6 ± 0.4	20.6 ± 0.4	20.0 ± 0.6	—	—
Brain						
Absolute	0.454 ± 0.007	0.456 ± 0.007	0.466 ± 0.007	0.438 ± 0.011	—	—
Relative	21.08 ± 0.54	21.15 ± 0.60	22.65 ± 0.51	21.94 ± 0.58	—	—
Heart						
Absolute	0.100 ± 0.005	0.108 ± 0.004	0.104 ± 0.005	0.110 ± 0.006	—	—
Relative	4.62 ± 0.17	5.01 ± 0.23	5.04 ± 0.19	5.53 ± 0.40	—	—
R. Kidney						
Absolute	0.154 ± 0.007	0.160 ± 0.006	0.156 ± 0.004	0.152 ± 0.006	—	—
Relative	7.12 ± 0.18	7.42 ± 0.35	7.58 ± 0.24	7.62 ± 0.36	—	—
Liver						
Absolute	1.066 ± 0.061	1.048 ± 0.041	0.946 ± 0.028	0.930 ± 0.027	—	—
Relative	49.32 ± 2.06	48.65 ± 2.40	45.92 ± 1.03	46.53 ± 0.90	—	—
Lung						
Absolute	0.146 ± 0.005	0.144 ± 0.009	0.148 ± 0.007	0.152 ± 0.007	—	—
Relative	6.79 ± 0.31	6.68 ± 0.44	7.21 ± 0.44	7.61 ± 0.34	—	—
R. Ovary						
Absolute	0.016 ± 0.002	0.012 ± 0.002	0.010 ± 0.001	0.012 ± 0.002	—	—
Relative	0.72 ± 0.07	0.54 ± 0.08	0.47 ± 0.05	0.62 ± 0.10	—	—
Thymus						
Absolute	0.065 ± 0.005	0.066 ± 0.002	0.068 ± 0.001	0.055 ± 0.003**	—	—
Relative	3.02 ± 0.17	3.05 ± 0.09	3.31 ± 0.03	2.77 ± 0.13**	—	—
Uterus						
Absolute	0.156 ± 0.011	0.117 ± 0.016	0.103 ± 0.011	0.172 ± 0.010**	—	—
Relative	7.22 ± 0.37	5.48 ± 0.80	4.98 ± 0.49	8.61 ± 0.48**	—	—

* Significantly different ($P \leq 0.05$) from the twice-daily administration group by a *t*-test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] All females in the 400 mg/kg once-daily group died before the end of the study. This high-dose comparison was not done.

TABLE F9
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Feed Study of Theophylline[a]

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Male				
n	10	10	10	10
Necropsy body wt	34.4 ± 1.0	31.4 ± 0.4**	29.7 ± 0.5**	29.5 ± 0.3**
Brain				
Absolute	0.456 ± 0.005	0.471 ± 0.004	0.473 ± 0.007	0.463 ± 0.006
Relative	13.34 ± 0.40	15.03 ± 0.19**	15.95 ± 0.34**	15.72 ± 0.27**
Heart				
Absolute	0.146 ± 0.004	0.152 ± 0.003	0.143 ± 0.003	0.130 ± 0.004**
Relative	4.26 ± 0.13	4.85 ± 0.10**	4.81 ± 0.10**	4.40 ± 0.10
R. Kidney				
Absolute	0.277 ± 0.011	0.304 ± 0.007	0.286 ± 0.010	0.273 ± 0.007
Relative	8.05 ± 0.23	9.70 ± 0.21**	9.60 ± 0.23**	9.25 ± 0.21**
Liver				
Absolute	1.430 ± 0.063	1.425 ± 0.038	1.323 ± 0.041	1.297 ± 0.033
Relative	41.48 ± 1.20	45.50 ± 1.33	44.47 ± 1.17	43.98 ± 1.06
Lung				
Absolute	0.159 ± 0.005	0.169 ± 0.006	0.178 ± 0.006	0.178 ± 0.006
Relative	4.64 ± 0.16	5.40 ± 0.21**	5.98 ± 0.16**	6.03 ± 0.17**
R. Testis				
Absolute	0.116 ± 0.001	0.121 ± 0.001	0.116 ± 0.003	0.115 ± 0.001
Relative	3.40 ± 0.10	3.85 ± 0.04**	3.92 ± 0.11**	3.90 ± 0.06**
Thymus				
Absolute	0.037 ± 0.003	0.032 ± 0.002	0.033 ± 0.001	0.033 ± 0.001
Relative	1.06 ± 0.07	1.03 ± 0.06	1.09 ± 0.04	1.13 ± 0.05

TABLE F9
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Feed Study of Theophylline (continued)

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Female				
n	10	10	10	10
Necropsy body wt	30.0 ± 0.6	27.8 ± 0.6*	28.0 ± 0.4*	27.9 ± 0.3*
Brain				
Absolute	0.477 ± 0.004	0.483 ± 0.003	0.484 ± 0.004	0.474 ± 0.003
Relative	15.98 ± 0.32	17.45 ± 0.34**	17.34 ± 0.23**	17.04 ± 0.28*
Heart				
Absolute	0.130 ± 0.004	0.130 ± 0.004	0.131 ± 0.005	0.125 ± 0.003
Relative	4.34 ± 0.12	4.71 ± 0.23	4.69 ± 0.19	4.49 ± 0.12
R. Kidney				
Absolute	0.206 ± 0.004	0.201 ± 0.005	0.212 ± 0.003	0.210 ± 0.005
Relative	6.89 ± 0.11	7.26 ± 0.21	7.59 ± 0.09**	7.54 ± 0.20**
Liver				
Absolute	1.383 ± 0.042	1.277 ± 0.044	1.351 ± 0.054	1.296 ± 0.025
Relative	46.26 ± 1.38	45.94 ± 1.14	48.30 ± 1.71	46.49 ± 0.64
Lung				
Absolute	0.166 ± 0.005	0.166 ± 0.004	0.181 ± 0.008	0.185 ± 0.005*
Relative	5.55 ± 0.14	6.00 ± 0.19	6.47 ± 0.25**	6.64 ± 0.18**
R. Ovary				
Absolute	0.013 ± 0.001	0.012 ± 0.001[b]	0.013 ± 0.001	0.013 ± 0.001
Relative	0.43 ± 0.03	0.44 ± 0.04[b]	0.46 ± 0.03	0.46 ± 0.04
Thymus				
Absolute	0.053 ± 0.003	0.044 ± 0.002**	0.043 ± 0.001**	0.041 ± 0.002**
Relative	1.75 ± 0.09	1.59 ± 0.04	1.55 ± 0.04*	1.48 ± 0.06**
Uterus				
Absolute	0.161 ± 0.010	0.157 ± 0.011	0.143 ± 0.010	0.153 ± 0.013
Relative	5.36 ± 0.27	5.69 ± 0.43	5.10 ± 0.32	5.47 ± 0.43

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

TABLE F10
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study of Theophylline[a]

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Male				
n	10	9	10	7
Necropsy body wt	37.9 ± 1.4	35.6 ± 1.2	33.6 ± 0.6**	33.1 ± 0.8**
Brain				
Absolute	0.462 ± 0.004	0.466 ± 0.008	0.459 ± 0.006	0.473 ± 0.005
Relative	12.32 ± 0.40	13.15 ± 0.38	13.68 ± 0.30*	14.33 ± 0.40**
Heart				
Absolute	0.148 ± 0.002	0.157 ± 0.011	0.144 ± 0.003	0.147 ± 0.005
Relative	3.94 ± 0.12	4.38 ± 0.23	4.29 ± 0.07	4.46 ± 0.22
R. Kidney				
Absolute	0.311 ± 0.011	0.289 ± 0.010	0.294 ± 0.006	0.274 ± 0.009*
Relative	8.25 ± 0.27	8.12 ± 0.20	8.74 ± 0.09	8.27 ± 0.14
Liver				
Absolute	1.497 ± 0.064	1.378 ± 0.069	1.331 ± 0.044	1.404 ± 0.034
Relative	39.59 ± 1.27	38.56 ± 1.30	39.53 ± 0.89	42.45 ± 0.92
Lung				
Absolute	0.170 ± 0.006	0.187 ± 0.017	0.169 ± 0.005	0.209 ± 0.014
Relative	4.53 ± 0.21	5.24 ± 0.44	5.03 ± 0.13	6.32 ± 0.48**
R. Testis				
Absolute	0.119 ± 0.001	0.113 ± 0.002*	0.117 ± 0.002	0.110 ± 0.002**
Relative	3.18 ± 0.09	3.20 ± 0.11	3.48 ± 0.04*	3.35 ± 0.10
Thymus				
Absolute	0.035 ± 0.001	0.034 ± 0.002	0.033 ± 0.001	0.033 ± 0.003
Relative	0.93 ± 0.04	0.94 ± 0.05	0.97 ± 0.04	0.98 ± 0.09

TABLE F10
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study of Theophylline
(continued)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Female				
n	9	10	10	0
Necropsy body wt	30.4 ± 0.6	27.9 ± 0.4*	29.0 ± 0.8	—[b]
Brain				
Absolute	0.462 ± 0.004	0.468 ± 0.009	0.467 ± 0.008	—
Relative	15.24 ± 0.38	16.79 ± 0.29*	16.19 ± 0.51	—
Heart				
Absolute	0.126 ± 0.002	0.128 ± 0.005	0.127 ± 0.006	—
Relative	4.14 ± 0.12	4.59 ± 0.16	4.39 ± 0.19	—
R. Kidney				
Absolute	0.196 ± 0.003	0.197 ± 0.004	0.192 ± 0.006	—
Relative	6.45 ± 0.21	7.07 ± 0.15	6.63 ± 0.21	—
Liver				
Absolute	1.339 ± 0.048	1.131 ± 0.027**	1.254 ± 0.056	—
Relative	44.08 ± 1.63	40.58 ± 0.92	43.10 ± 1.20	—
Lung				
Absolute	0.158 ± 0.005	0.164 ± 0.006	0.162 ± 0.007	—
Relative	5.20 ± 0.18	5.88 ± 0.22	5.59 ± 0.23	—
R. Ovary				
Absolute	0.013 ± 0.001	0.012 ± 0.001[c]	0.012 ± 0.001	—
Relative	0.42 ± 0.03	0.42 ± 0.03[c]	0.40 ± 0.03	—
Thymus				
Absolute	0.045 ± 0.002	0.047 ± 0.002	0.044 ± 0.002	—
Relative	1.49 ± 0.06	1.67 ± 0.07	1.53 ± 0.06	—
Uterus				
Absolute	0.161 ± 0.015	0.148 ± 0.009	0.151 ± 0.012	—
Relative	5.28 ± 0.44	5.28 ± 0.29	5.21 ± 0.42	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] All animals died before the end of the study.
[c] n=9

APPENDIX G
HEMATOLOGY RESULTS

TABLE G1
Hematology Data for Rats in the 16-Day Feed Study of Theophylline[a]

	0 ppm	500 ppm	1,000 ppm	2,000 ppm	4,000 ppm	8,000 ppm
Male						
n	4	5	5	5	5	3
Hematocrit (%)	43.9 ± 0.9	44.5 ± 0.2	44.0 ± 0.3	46.7 ± 0.3**	48.0 ± 0.8**	50.1 ± 1.2**
Hemoglobin (g/dL)	15.3 ± 0.4	16.1 ± 0.1	15.9 ± 0.2	16.8 ± 0.1**	17.4 ± 0.3**	18.4 ± 0.5**
Erythrocytes (10^6/µL)	6.83 ± 0.12	6.95 ± 0.02	6.70 ± 0.14	7.27 ± 0.08*	7.29 ± 0.11*	8.01 ± 0.24**
Leukocytes (10^3/µL)	9.38 ± 0.52	8.62 ± 0.32	10.48 ± 0.35	8.00 ± 0.40	9.16 ± 0.82	8.00 ± 0.10
Female						
n	4	5	4	4	4	5
Hematocrit (%)	44.1 ± 0.6	49.7 ± 0.3*	45.4 ± 1.6	49.0 ± 0.4*	51.2 ± 0.4**	51.0 ± 2.7**
Hemoglobin (g/dL)	15.7 ± 0.3	17.8 ± 0.1*	16.5 ± 0.4	18.0 ± 0.1**	18.3 ± 0.1**	18.0 ± 1.0**
Erythrocytes (10^6/µL)	7.19 ± 0.12	7.51 ± 0.06	6.86 ± 0.39	7.54 ± 0.13	7.88 ± 0.11	7.53 ± 0.50
Leukocytes (10^3/µL)	5.78 ± 0.23	6.14 ± 0.67	5.35 ± 1.12	6.45 ± 0.82	7.63 ± 0.28	5.80 ± 0.16

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE G2
Hematology Data for Rats in the 16-Day Gavage Study of Theophylline[a]:
Comparison of Groups Receiving Once-Daily Administration

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
n	5	5	5	5	5	0
Male						
Hematocrit (%)	36.8 ± 0.5	35.9 ± 0.4	36.4 ± 0.2	36.9 ± 0.3	38.8 ± 0.8	—[b]
Hemoglobin (g/dL)	14.6 ± 0.2	14.3 ± 0.2	14.7 ± 0.1	14.8 ± 0.1	15.2 ± 0.4*	—
Erythrocytes (10^6/µL)	8.29 ± 0.09	8.15 ± 0.14	8.41 ± 0.05	8.28 ± 0.09	8.58 ± 0.21	—
Reticulocytes (10^6/µL)	0.27 ± 0.01	0.22 ± 0.02	0.22 ± 0.01	0.23 ± 0.02	0.22 ± 0.02	—
Mean cell volume (fL)	44.6 ± 0.4	44.0 ± 0.4	43.4 ± 0.2	44.4 ± 0.2	45.2 ± 0.2	—
Mean cell hemoglobin (pg)	17.6 ± 0.1	17.6 ± 0.2	17.5 ± 0.1	17.8 ± 0.1	17.7 ± 0.1	—
Mean cell hemoglobin concentration (g/dL)	39.6 ± 0.5	39.9 ± 0.3	40.3 ± 0.1	40.0 ± 0.2	39.3 ± 0.3	—
Platelets (10^3/µL)	734.8 ± 20.7	738.2 ± 24.4	734.8 ± 25.5	704.2 ± 19.4	712.2 ± 18.0	—
Leukocytes (10^3/µL)	4.06 ± 0.36	3.92 ± 0.27	3.62 ± 0.24	4.06 ± 0.36	3.42 ± 0.32	—
Segmented neutrophils (10^3/µL)	0.54 ± 0.05	0.51 ± 0.04	0.42 ± 0.05	0.59 ± 0.07	0.54 ± 0.06	—
Bands (10^3/µL)	0.04 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.04 ± 0.03	0.04 ± 0.02	—
Lymphocytes (10^3/µL)	3.34 ± 0.35	3.26 ± 0.22	3.08 ± 0.22	3.30 ± 0.35	2.72 ± 0.30	—
Monocytes (10^3/µL)	0.04 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.05 ± 0.02	—
Eosinophils (10^3/µL)	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	—
Female						
Hematocrit (%)	28.1 ± 0.3	28.6 ± 0.6	28.9 ± 0.2	27.9 ± 0.1	30.5 ± 0.5*	—
Hemoglobin (g/dL)	12.1 ± 0.1	12.3 ± 0.2	12.4 ± 0.2	12.0 ± 0.1	12.9 ± 0.1*	—
Erythrocytes (10^6/µL)	6.67 ± 0.08	6.68 ± 0.16	6.80 ± 0.07	6.61 ± 0.05	7.09 ± 0.08*	—
Reticulocytes (10^6/µL)	0.19 ± 0.02	0.18 ± 0.01	0.20 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	—
Mean cell volume (fL)	42.0 ± 0.3	42.8 ± 0.5	42.4 ± 0.2	42.0 ± 0.3	43.0 ± 0.4	—
Mean cell hemoglobin (pg)	18.2 ± 0.2	18.4 ± 0.2	18.2 ± 0.1	18.2 ± 0.2	18.2 ± 0.1	—
Mean cell hemoglobin concentration (g/dL)	43.2 ± 0.5	43.0 ± 0.4	42.7 ± 0.4	43.2 ± 0.3	42.3 ± 0.5	—
Platelets (10^3/µL)	785.2 ± 14.0	672.2 ± 32.5	753.2 ± 41.8	707.0 ± 7.9	839.4 ± 21.1	—
Leukocytes (10^3/µL)	3.36 ± 0.27	2.94 ± 0.35	3.74 ± 0.25	3.70 ± 0.29	3.56 ± 0.16	—
Segmented neutrophils (10^3/µL)	0.34 ± 0.09	0.47 ± 0.03	0.45 ± 0.07	0.47 ± 0.07	0.41 ± 0.07	—
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—
Lymphocytes (10^3/µL)	2.99 ± 0.29	2.43 ± 0.32	3.24 ± 0.23	3.19 ± 0.24	3.13 ± 0.12	—
Monocytes (10^3/µL)	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	—
Eosinophils (10^3/µL)	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] All animals died before the end of the study.

TABLE G3
Hematology Data for Rats in the 16-Day Gavage Study of Theophylline[a]: Comparisons of Once-Daily to Twice-Daily Administration

	Low-dose Comparison		Mid-dose Comparison		High-dose Comparison[b]	
	12.5 mg/kg twice daily	25 mg/kg once daily	50 mg/kg twice daily	100 mg/kg once daily	200 mg/kg twice daily	400 mg/kg once daily
Male						
n	5	5	5	5	0	0
Hematocrit (%)	35.9 ± 0.7	35.9 ± 0.4	36.5 ± 0.4	36.9 ± 0.3	—	—
Hemoglobin (g/dL)	14.3 ± 0.3	14.3 ± 0.2	14.6 ± 0.2	14.8 ± 0.1	—	—
Erythrocytes (10^6/µL)	8.02 ± 0.07	8.15 ± 0.14	8.13 ± 0.11	8.28 ± 0.09	—	—
Reticulocytes (10^6/µL)	0.23 ± 0.01	0.22 ± 0.02	0.26 ± 0.02	0.23 ± 0.02	—	—
Mean cell volume (fL)	44.8 ± 0.9	44.0 ± 0.4	44.8 ± 0.2	44.4 ± 0.2	—	—
Mean cell hemoglobin (pg)	17.8 ± 0.4	17.6 ± 0.2	17.9 ± 0.2	17.8 ± 0.1	—	—
Mean cell hemoglobin concentration (g/dL)	39.8 ± 0.2	39.9 ± 0.3	39.9 ± 0.4	40.0 ± 0.2	—	—
Platelets (10^3/µL)	697.0 ± 24.5	738.2 ± 24.4	726.6 ± 7.8	704.2 ± 19.4	—	—
Leukocytes (10^3/µL)	4.10 ± 0.26	3.92 ± 0.27	3.48 ± 0.22	4.06 ± 0.36	—	—
Segmented neutrophils (10^3/µL)	0.75 ± 0.16	0.51 ± 0.04	0.48 ± 0.07	0.59 ± 0.07	—	—
Bands (10^3/µL)	0.08 ± 0.03	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.03	—	—
Lymphocytes (10^3/µL)	3.10 ± 0.27	3.26 ± 0.22	2.92 ± 0.23	3.30 ± 0.35	—	—
Monocytes (10^3/µL)	0.05 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	—	—
Eosinophils (10^3/µL)	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	—	—
Female						
n	5	5	5	5	1	0
Hematocrit (%)	28.5 ± 0.3	28.6 ± 0.6	28.4 ± 0.2	27.9 ± 0.1	—	—
Hemoglobin (g/dL)	12.5 ± 0.2	12.3 ± 0.2	12.1 ± 0.1	12.0 ± 0.1	—	—
Erythrocytes (10^6/µL)	6.81 ± 0.13	6.68 ± 0.16	6.52 ± 0.11	6.61 ± 0.05	—	—
Reticulocytes (10^6/µL)	0.17 ± 0.02	0.18 ± 0.01	0.17 ± 0.02	0.19 ± 0.02	—	—
Mean cell volume (fL)	41.8 ± 0.6	42.8 ± 0.5	43.8 ± 0.8	42.0 ± 0.3	—	—
Mean cell hemoglobin (pg)	18.4 ± 0.1	18.4 ± 0.2	18.6 ± 0.3	18.2 ± 0.2	—	—
Mean cell hemoglobin concentration (g/dL)	43.8 ± 0.5	43.0 ± 0.4	42.7 ± 0.2	43.2 ± 0.3	—	—
Platelets (10^3/µL)	743.8 ± 17.7	672.2 ± 32.5	779.2 ± 29.5	707.0 ± 7.9*	—	—
Leukocytes (10^3/µL)	3.50 ± 0.28	2.94 ± 0.35	3.74 ± 0.35	3.70 ± 0.29	—	—
Segmented neutrophils (10^3/µL)	0.57 ± 0.14	0.47 ± 0.03	0.60 ± 0.09	0.47 ± 0.07	—	—
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—	—
Lymphocytes (10^3/µL)	2.89 ± 0.19	2.43 ± 0.32	3.12 ± 0.26	3.19 ± 0.24	—	—
Monocytes (10^3/µL)	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	—	—
Eosinophils (10^3/µL)	0.04 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	—	—

* Significantly different ($P \leq 0.05$) from the twice-daily administration group by the Wilcoxon rank sum test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] All animals in the 400 mg/kg once-daily group and all but one female in the 200 mg/kg twice-daily group died before the end of the study. These high-dose comparisons were not done.

TABLE G4
Hematology Data for Rats in the 14-Week Feed Study of Theophylline[a]

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Male				
n	10	10	9	9
Hematocrit (%)	47.4 ± 0.4	47.3 ± 0.6	47.3 ± 0.7	48.3 ± 0.8
Hemoglobin (g/dL)	15.8 ± 0.2	15.7 ± 0.2	15.7 ± 0.3	16.0 ± 0.3
Erythrocytes (10^6/µL)	9.30 ± 0.08	9.21 ± 0.12	9.04 ± 0.13	8.90 ± 0.14
Reticulocytes (10^6/µL)	0.20 ± 0.02	0.18 ± 0.01	0.14 ± 0.01*	0.15 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	0.15 ± 0.06
Mean cell volume (fL)	50.9 ± 0.2	51.3 ± 0.2	52.3 ± 0.2**	54.3 ± 0.2**
Mean cell hemoglobin (pg)	17.0 ± 0.1	17.0 ± 0.1	17.4 ± 0.1**	18.0 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	33.2 ± 0.2	33.2 ± 0.1	33.2 ± 0.1	33.2 ± 0.2
Platelets (10^3/µL)	686.1 ± 32.0	674.0 ± 31.4	791.7 ± 44.2	917.7 ± 41.7**
Leukocytes (10^3/µL)	10.45 ± 0.42	11.06 ± 0.35	10.74 ± 0.68	11.07 ± 0.67
Segmented neutrophils (10^3/µL)	1.82 ± 0.13	1.89 ± 0.27	1.70 ± 0.16	2.49 ± 0.19
Bands (10^3/µL)	0.09 ± 0.04	0.09 ± 0.03	0.03 ± 0.02	0.08 ± 0.02
Lymphocytes (10^3/µL)	8.16 ± 0.44	8.72 ± 0.40	8.84 ± 0.67	8.29 ± 0.62
Atypical lymphocytes (10^3/µL)	0.10 ± 0.04	0.13 ± 0.05	0.01 ± 0.01	0.07 ± 0.05
Monocytes (10^3/µL)	0.13 ± 0.03	0.11 ± 0.01	0.08 ± 0.05*	0.07 ± 0.02*
Eosinophils (10^3/µL)	0.14 ± 0.04	0.13 ± 0.03	0.09 ± 0.03	0.06 ± 0.02
Female				
n	10	10	10	10
Hematocrit (%)	46.3 ± 0.5	47.7 ± 0.8	47.3 ± 0.7	48.4 ± 0.7
Hemoglobin (g/dL)	15.5 ± 0.2	15.6 ± 0.3	15.7 ± 0.3	16.0 ± 0.3
Erythrocytes (10^6/µL)	8.43 ± 0.09	8.63 ± 0.14	8.54 ± 0.12	8.73 ± 0.14
Reticulocytes (10^6/µL)	0.16 ± 0.01	0.21 ± 0.03[b]	0.18 ± 0.02	0.15 ± 0.01[b]
Nucleated erythrocytes (10^3/µL)	0.02 ± 0.02	0.07 ± 0.03	0.02 ± 0.01	0.04 ± 0.03
Mean cell volume (fL)	54.9 ± 0.1	55.4 ± 0.2	55.4 ± 0.2	55.5 ± 0.2*
Mean cell hemoglobin (pg)	18.4 ± 0.1	18.1 ± 0.1*	18.4 ± 0.1	18.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.5 ± 0.1	32.7 ± 0.2*	33.3 ± 0.2	33.1 ± 0.2
Platelets (10^3/µL)	687.0 ± 43.4	775.8 ± 59.2	678.2 ± 20.3	790.2 ± 70.8
Leukocytes (10^3/µL)	8.31 ± 0.38	11.95 ± 0.66**	10.10 ± 0.65	11.05 ± 1.09
Segmented neutrophils (10^3/µL)	1.18 ± 0.11	1.99 ± 0.25*	1.72 ± 0.19*	2.45 ± 0.35**
Bands (10^3/µL)	0.04 ± 0.02	0.06 ± 0.02	0.07 ± 0.03	0.12 ± 0.05
Lymphocytes (10^3/µL)	6.90 ± 0.33	9.62 ± 0.51**	8.12 ± 0.52	8.19 ± 0.96
Atypical lymphocytes (10^3/µL)	0.06 ± 0.02	0.09 ± 0.06	0.03 ± 0.03	0.08 ± 0.05
Monocytes (10^3/µL)	0.07 ± 0.02	0.11 ± 0.03	0.09 ± 0.04	0.10 ± 0.03
Eosinophils (10^3/µL)	0.05 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.11 ± 0.04

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9

TABLE G5
Hematology Data for Rats in the 14-Week Gavage Study of Theophylline[a]

	Vehicle Control	37.5 mg/kg	75 mg/kg	150 mg/kg
n	10	10	10	9
Male				
Hematocrit (%)	47.9 ± 0.5	48.2 ± 0.8	47.6 ± 0.7	48.2 ± 0.9
Hemoglobin (g/dL)	15.6 ± 0.2	15.8 ± 0.2	15.6 ± 0.2	16.0 ± 0.2
Erythrocytes (10^6/µL)	9.39 ± 0.08	9.37 ± 0.11	9.21 ± 0.11	9.21 ± 0.15
Reticulocytes (10^6/µL)	0.44 ± 0.02	0.44 ± 0.02	0.45 ± 0.02	0.47 ± 0.03
Mean cell volume (fL)	50.9 ± 0.2	51.6 ± 0.3	51.5 ± 0.2	52.6 ± 0.2**
Mean cell hemoglobin (pg)	16.6 ± 0.1	16.9 ± 0.1**	16.9 ± 0.1**	17.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.6 ± 0.1	32.9 ± 0.2	32.7 ± 0.1	33.2 ± 0.2
Platelets (10^3/µL)	651.0 ± 24.4	668.9 ± 28.9	684.9 ± 46.3	640.1 ± 36.0
Leukocytes (10^3/µL)	12.01 ± 0.44	13.26 ± 0.71	12.61 ± 0.59	12.58 ± 0.73
Segmented neutrophils (10^3/µL)	2.00 ± 0.30	1.93 ± 0.11	1.72 ± 0.17	2.12 ± 0.36
Lymphocytes (10^3/µL)	9.64 ± 0.26	11.08 ± 0.66	10.54 ± 0.46	10.19 ± 0.60
Monocytes (10^3/µL)	0.29 ± 0.08	0.13 ± 0.06	0.20 ± 0.06	0.14 ± 0.04
Eosinophils (10^3/µL)	0.05 ± 0.03	0.10 ± 0.05	0.09 ± 0.04	0.08 ± 0.03
Female				
Hematocrit (%)	46.7 ± 0.6	45.7 ± 0.5	46.8 ± 0.4	47.5 ± 0.7
Hemoglobin (g/dL)	15.2 ± 0.2	15.0 ± 0.2	15.3 ± 0.2	15.5 ± 0.2
Erythrocytes (10^6/µL)	8.49 ± 0.10	8.34 ± 0.09	8.38 ± 0.08	8.63 ± 0.13
Reticulocytes (10^6/µL)	0.42 ± 0.02	0.41 ± 0.02	0.47 ± 0.03	0.48 ± 0.02
Mean cell volume (fL)	55.0 ± 0.2	54.7 ± 0.2	55.7 ± 0.2	55.0 ± 0.3
Mean cell hemoglobin (pg)	18.0 ± 0.1	18.0 ± 0.1	18.2 ± 0.1	17.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.2	32.8 ± 0.2	32.6 ± 0.2	32.5 ± 0.2
Platelets (10^3/µL)	778.2 ± 58.9	727.0 ± 18.1	861.3 ± 44.1	804.8 ± 61.0
Leukocytes (10^3/µL)	10.20 ± 0.27	9.94 ± 0.69	11.97 ± 0.65	12.38 ± 1.12
Segmented neutrophils (10^3/µL)	1.37 ± 0.23	1.38 ± 0.21	1.51 ± 0.14	1.63 ± 0.18
Lymphocytes (10^3/µL)	8.60 ± 0.18	8.27 ± 0.51	10.11 ± 0.58	10.50 ± 0.99
Monocytes (10^3/µL)	0.18 ± 0.05	0.16 ± 0.04	0.24 ± 0.13	0.15 ± 0.09
Eosinophils (10^3/µL)	0.04 ± 0.03	0.09 ± 0.03	0.05 ± 0.04	0.08 ± 0.02

** Significantly different ($P \leq 0.01$) from the vehicle control group by Dunn's or Shirley's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE G6
Hematology Data for Mice in the 16-Day Feed Study of Theophylline[a]

	0 ppm	500 ppm	1,000 ppm	2,000 ppm	4,000 ppm	8,000 ppm
Male						
n	4	4	5	5	5	5
Hematocrit (%)	38.7 ± 0.9	35.2 ± 1.9	38.0 ± 1.4	39.0 ± 0.8	43.2 ± 0.7*	41.2 ± 1.8
Hemoglobin (g/dL)	12.1 ± 0.3	11.6 ± 0.6	12.6 ± 0.5	12.6 ± 0.3	14.2 ± 0.2**	13.5 ± 0.6*
Erythrocytes (10^6/µL)	6.61 ± 0.33	5.90 ± 0.25	6.41 ± 0.27	6.51 ± 0.09	7.25 ± 0.13	6.93 ± 0.32
Leukocytes (10^3/µL)	1.43 ± 0.31	0.75 ± 0.10	1.16 ± 0.14	1.22 ± 0.16	1.10 ± 0.27	0.88 ± 0.11
Female						
n	4	5	5	5	5	5
Hematocrit (%)	39.5 ± 0.6	36.8 ± 0.9	38.0 ± 0.6	32.6 ± 3.6	37.4 ± 1.5	37.2 ± 1.6
Hemoglobin (g/dL)	13.0 ± 0.1	12.6 ± 0.4	13.1 ± 0.2	11.2 ± 1.2	12.7 ± 0.7	12.4 ± 0.5
Erythrocytes (10^6/µL)	6.61 ± 0.04	6.33 ± 0.16	6.63 ± 0.10	5.55 ± 0.58	6.45 ± 0.29	6.40 ± 0.27
Leukocytes (10^3/µL)	1.03 ± 0.21	1.08 ± 0.15	0.92 ± 0.23	0.60 ± 0.13	0.70 ± 0.21	0.50 ± 0.09

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE G7
Hematology Data for Mice in the 16-Day Gavage Study of Theophylline[a]:
Comparison of Groups Receiving Once-Daily Administration

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Male						
n	4	5	5	5	4	2
Hematocrit (%)	36.3 ± 0.2	34.6 ± 0.7	35.6 ± 0.5	34.2 ± 3.1	36.2 ± 0.4	36.8 ± 1.0
Hemoglobin (g/dL)	15.6 ± 0.1	15.2 ± 0.3	15.5 ± 0.1	14.8 ± 1.3	15.6 ± 0.1	16.0 ± 0.5
Erythrocytes (10^6/µL)	9.28 ± 0.08	9.02 ± 0.16	9.20 ± 0.12	8.72 ± 0.72	9.19 ± 0.09	9.23 ± 0.29
Reticulocytes (10^6/µL)	0.20 ± 0.03	0.20 ± 0.03	0.20 ± 0.03	0.22 ± 0.03	0.28 ± 0.03	0.20 ± 0.01
Mean cell volume (fL)	39.3 ± 0.3	38.4 ± 0.2	39.0 ± 0.3	39.2 ± 0.5	39.5 ± 0.6	40.0 ± 0.0
Mean cell hemoglobin (pg)	16.8 ± 0.1	16.9 ± 0.1	16.8 ± 0.1	17.0 ± 0.2	17.0 ± 0.2	17.3 ± 0.0
Mean cell hemoglobin concentration (g/dL)	43.0 ± 0.4	44.1 ± 0.2	43.4 ± 0.3	43.4 ± 0.3	43.1 ± 0.4	43.5 ± 0.2
Platelets (10^3/µL)	782.3 ± 18.7	804.3 ± 42.9[b]	789.8 ± 17.8	678.4 ± 61.6	687.0 ± 62.2[c]	749.0 ± 4.0
Leukocytes (10^3/µL)	1.85 ± 0.37	1.50 ± 0.15	1.14 ± 0.21	1.02 ± 0.12	1.00 ± 0.21	0.95 ± 0.35
Segmented neutrophils (10^3/µL)	0.24 ± 0.04	0.23 ± 0.05	0.12 ± 0.05	0.11 ± 0.02	0.15 ± 0.05	0.09 ± 0.02
Bands (10^3/µL)	0.05 ± 0.02	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.05 ± 0.02	0.02 ± 0.01
Lymphocytes (10^3/µL)	1.53 ± 0.31	1.21 ± 0.11	0.99 ± 0.18	0.89 ± 0.10	0.75 ± 0.15	0.83 ± 0.38
Monocytes (10^3/µL)	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Eosinophils (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Female						
n	5	5	4	5	5	0
Hematocrit (%)	36.3 ± 0.5	35.2 ± 1.9	36.0 ± 1.0	35.5 ± 0.3	35.9 ± 0.7	—[d]
Hemoglobin (g/dL)	15.3 ± 0.2	15.4 ± 0.5	15.5 ± 0.2	15.6 ± 0.1	15.9 ± 0.1	—
Erythrocytes (10^6/µL)	9.01 ± 0.19	8.92 ± 0.34	9.04 ± 0.19	9.10 ± 0.09	9.16 ± 0.04	—
Reticulocytes (10^6/µL)	0.24 ± 0.01	0.17 ± 0.02	0.21 ± 0.02	0.19 ± 0.01	0.25 ± 0.01	—
Mean cell volume (fL)	40.6 ± 0.5	39.4 ± 1.1	39.8 ± 0.9	38.8 ± 0.6	39.2 ± 0.6	—
Mean cell hemoglobin (pg)	17.0 ± 0.1	17.3 ± 0.2	17.1 ± 0.2	17.1 ± 0.2	17.3 ± 0.1	—
Mean cell hemoglobin concentration (g/dL)	42.3 ± 0.5	43.9 ± 1.3	43.0 ± 1.0	44.0 ± 0.6	44.2 ± 0.7	—
Platelets (10^3/µL)	784.4 ± 22.7	795.8 ± 40.3[b]	681.5 ± 70.7	774.4 ± 16.0	688.4 ± 63.4	—
Leukocytes (10^3/µL)	1.22 ± 0.20	1.18 ± 0.23	1.53 ± 0.41	1.22 ± 0.14	0.92 ± 0.16	—
Segmented neutrophils (10^3/µL)	0.18 ± 0.05	0.12 ± 0.03	0.14 ± 0.06	0.17 ± 0.02	0.12 ± 0.03	—
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	—
Lymphocytes (10^3/µL)	1.03 ± 0.18	1.03 ± 0.19	1.34 ± 0.35	1.04 ± 0.14	0.79 ± 0.13	—
Monocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	—
Eosinophils (10^3/µL)	0.00 ± 0.00	0.02 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	—

[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=4
[c] n=3
[d] All animals died before the end of the study.

TABLE G8
Hematology Data for Mice in the 16-Day Gavage Study of Theophylline[a]:
Comparisons of Once-Daily to Twice-Daily Administration

	Low-dose Comparison		Mid-dose Comparison		High-dose Comparison[b]	
	12.5 mg/kg twice daily	25 mg/kg once daily	50 mg/kg twice daily	100 mg/kg once daily	200 mg/kg twice daily	400 mg/kg once daily
Male						
n	5	5	5	5	5	2
Hematocrit (%)	36.7 ± 0.5	34.6 ± 0.7	36.3 ± 0.8	34.2 ± 3.1	37.0 ± 2.2	36.8 ± 1.0
Hemoglobin (g/dL)	15.9 ± 0.3	15.2 ± 0.3	15.9 ± 0.3	14.8 ± 1.3	16.1 ± 0.8	16.0 ± 0.5
Erythrocytes (10^6/µL)	9.42 ± 0.09	9.02 ± 0.16*	9.33 ± 0.21	8.72 ± 0.72	9.25 ± 0.40	9.23 ± 0.29
Reticulocytes (10^6/µL)	0.23 ± 0.01	0.20 ± 0.03	0.19 ± 0.01	0.22 ± 0.03	0.23 ± 0.03	0.20 ± 0.01
Mean cell volume (fL)	38.6 ± 0.2	38.4 ± 0.2	39.0 ± 0.0	39.2 ± 0.5	39.8 ± 0.9	40.0 ± 0.0
Mean cell hemoglobin (pg)	16.9 ± 0.2	16.9 ± 0.1	17.0 ± 0.1	17.0 ± 0.2	17.3 ± 0.2	17.3 ± 0.0
Mean cell hemoglobin concentration (g/dL)	43.4 ± 0.4	44.1 ± 0.2	43.8 ± 0.3	43.4 ± 0.3	43.5 ± 0.5	43.5 ± 0.2
Platelets (10^3/µL)	676.2 ± 42.1	804.3 ± 42.9[c]	782.0 ± 24.4	678.4 ± 61.6	646.6 ± 56.9	749.0 ± 4.0*
Leukocytes (10^3/µL)	1.64 ± 0.17	1.50 ± 0.15	1.58 ± 0.12	1.02 ± 0.12**	1.18 ± 0.21	0.95 ± 0.35
Segmented neutrophils (10^3/µL)	0.24 ± 0.03	0.23 ± 0.05	0.10 ± 0.02	0.11 ± 0.02	0.09 ± 0.02	0.09 ± 0.02
Bands (10^3/µL)	0.06 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.04 ± 0.02	0.02 ± 0.01
Lymphocytes (10^3/µL)	1.34 ± 0.17	1.21 ± 0.11	1.43 ± 0.14	0.89 ± 0.10*	1.03 ± 0.17	0.83 ± 0.38
Monocytes (10^3/µL)	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Eosinophils (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Female						
n	5	5	5	5	5	0
Hematocrit (%)	35.4 ± 0.9	35.2 ± 1.9	32.5 ± 2.5	35.5 ± 0.3	—	—
Hemoglobin (g/dL)	15.4 ± 0.3	15.4 ± 0.5	14.3 ± 1.0	15.6 ± 0.1	—	—
Erythrocytes (10^6/µL)	8.87 ± 0.16	8.92 ± 0.34	8.41 ± 0.59	9.10 ± 0.09	—	—
Reticulocytes (10^6/µL)	0.18 ± 0.01	0.17 ± 0.02	0.14 ± 0.02	0.19 ± 0.01*	—	—
Mean cell volume (fL)	40.0 ± 0.7	39.4 ± 1.1	38.4 ± 0.5	38.8 ± 0.6	—	—
Mean cell hemoglobin (pg)	17.3 ± 0.2	17.3 ± 0.2	17.0 ± 0.1	17.1 ± 0.2	—	—
Mean cell hemoglobin concentration (g/dL)	43.5 ± 0.8	43.9 ± 1.3	44.1 ± 0.5	44.0 ± 0.6	—	—
Platelets (10^3/µL)	701.0 ± 47.5	795.8 ± 40.3[c]	701.4 ± 53.3	774.4 ± 16.0	—	—
Leukocytes (10^3/µL)	1.08 ± 0.09	1.18 ± 0.23	1.34 ± 0.21	1.22 ± 0.14	—	—
Segmented neutrophils (10^3/µL)	0.13 ± 0.04	0.12 ± 0.03	0.09 ± 0.01	0.17 ± 0.02*	—	—
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	—	—
Lymphocytes (10^3/µL)	0.93 ± 0.08	1.03 ± 0.19	1.22 ± 0.20	1.04 ± 0.14	—	—
Monocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—	—
Eosinophils (10^3/µL)	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	—	—

* Significantly different ($P \leq 0.05$) from the twice-daily administration group by Wilcoxon rank sum test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] All females in the 400 mg/kg once-daily group died before the end of the study. The high-dose comparison of females was not done.
[c] n=4

TABLE G9
Hematology Data for Mice in the 14-Week Feed Study of Theophylline[a]

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
n	10	10	10	10
Male				
Hematocrit (%)	43.8 ± 0.5	43.5 ± 0.5	43.9 ± 0.3	44.9 ± 0.3
Hemoglobin (g/dL)	15.8 ± 0.2	15.6 ± 0.2	15.8 ± 0.1	16.2 ± 0.1
Erythrocytes (10^6/µL)	9.90 ± 0.11	9.83 ± 0.15	9.92 ± 0.12	10.12 ± 0.08
Reticulocytes (10^6/µL)	0.10 ± 0.03	0.14 ± 0.02	0.16 ± 0.03	0.15 ± 0.02
Mean cell volume (fL)	44.3 ± 0.4	44.3 ± 0.3	44.4 ± 0.3	44.4 ± 0.3
Mean cell hemoglobin (pg)	15.9 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	16.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	35.9 ± 0.2	35.9 ± 0.2	36.1 ± 0.2	36.2 ± 0.3
Platelets (10^3/µL)	1,013.2 ± 25.3	1,042.4 ± 31.6	1,056.1 ± 25.5	994.2 ± 24.8
Leukocytes (10^3/µL)	3.33 ± 0.41	3.22 ± 0.46	4.58 ± 0.38	5.93 ± 0.42**
Segmented neutrophils (10^3/µL)	0.70 ± 0.10	0.71 ± 0.12	1.01 ± 0.17	1.35 ± 0.15**
Bands (10^3/µL)	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.11 ± 0.03
Lymphocytes (10^3/µL)	2.57 ± 0.31	2.44 ± 0.40	3.41 ± 0.31	4.35 ± 0.33**
Monocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.02
Eosinophils (10^3/µL)	0.03 ± 0.01	0.04 ± 0.02	0.09 ± 0.03	0.09 ± 0.03
Female				
Hematocrit (%)	43.6 ± 0.5	43.0 ± 0.7	43.5 ± 0.7	45.0 ± 1.0
Hemoglobin (g/dL)	15.7 ± 0.2	15.6 ± 0.3	15.7 ± 0.2	16.4 ± 0.4
Erythrocytes (10^6/µL)	9.77 ± 0.12	9.62 ± 0.20	9.65 ± 0.19	10.13 ± 0.24
Reticulocytes (10^6/µL)	0.19 ± 0.02	0.11 ± 0.02	0.12 ± 0.02	0.19 ± 0.03
Mean cell volume (fL)	44.7 ± 0.3	44.8 ± 0.5	45.1 ± 0.4	44.4 ± 0.4
Mean cell hemoglobin (pg)	16.0 ± 0.1	16.2 ± 0.1	16.3 ± 0.1	16.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	35.9 ± 0.2	36.3 ± 0.3	36.1 ± 0.2	36.6 ± 0.2
Platelets (10^3/µL)	887.9 ± 35.6	973.0 ± 21.8	997.6 ± 44.8	947.1 ± 49.2
Leukocytes (10^3/µL)	3.35 ± 0.28	4.31 ± 0.36	5.01 ± 0.31**	4.81 ± 0.44**
Segmented neutrophils (10^3/µL)	0.48 ± 0.08	0.60 ± 0.10	1.21 ± 0.17**	1.16 ± 0.18**
Bands (10^3/µL)	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.01	0.07 ± 0.01
Lymphocytes (10^3/µL)	2.78 ± 0.24	3.56 ± 0.31	3.63 ± 0.22	3.52 ± 0.32
Monocytes (10^3/µL)	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Eosinophils (10^3/µL)	0.06 ± 0.01	0.10 ± 0.02	0.13 ± 0.03	0.06 ± 0.01

** Significantly different ($P \leq 0.01$) from the control group by Shirley's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE G10
Hematology Data for Mice in the 14-Week Gavage Study of Theophylline[a]

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Male				
n	10	9	10	7
Hematocrit (%)	44.5 ± 0.5	45.9 ± 0.9	45.8 ± 0.5	43.9 ± 0.9
Hemoglobin (g/dL)	15.4 ± 0.2	16.0 ± 0.3	15.8 ± 0.2	15.1 ± 0.3
Erythrocytes (10^6/µL)	9.70 ± 0.12	10.01 ± 0.20	9.89 ± 0.10	9.23 ± 0.21
Reticulocytes (10^6/µL)	0.24 ± 0.02	0.20 ± 0.02	0.22 ± 0.02	0.23 ± 0.05
Mean cell volume (fL)	45.8 ± 0.2	46.0 ± 0.2	46.3 ± 0.2	47.3 ± 0.2**
Mean cell hemoglobin (pg)	15.8 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	16.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	34.5 ± 0.1	34.8 ± 0.2	34.5 ± 0.1	34.4 ± 0.2
Platelets (10^3/µL)	976.1 ± 28.2	1,028.9 ± 42.8	1,030.2 ± 33.7	1,087.1 ± 49.3
Leukocytes (10^3/µL)	3.65 ± 0.53	3.68 ± 0.32	3.89 ± 0.39	3.67 ± 0.60
Segmented neutrophils (10^3/µL)	0.70 ± 0.12	0.51 ± 0.09	0.73 ± 0.10	0.94 ± 0.16
Lymphocytes (10^3/µL)	2.95 ± 0.44	3.15 ± 0.26	3.16 ± 0.32	2.69 ± 0.43
Monocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Female				
n	9	10	10	0
Hematocrit (%)	44.3 ± 0.2	44.6 ± 0.3	44.7 ± 0.5	—[b]
Hemoglobin (g/dL)	15.3 ± 0.1	15.4 ± 0.1	15.6 ± 0.2	—
Erythrocytes (10^6/µL)	9.53 ± 0.05	9.56 ± 0.05	9.52 ± 0.10	—
Reticulocytes (10^6/µL)	0.20 ± 0.01	0.20 ± 0.02	0.18 ± 0.02[c]	—
Mean cell volume (fL)	46.3 ± 0.3	46.7 ± 0.2	46.9 ± 0.2	—
Mean cell hemoglobin (pg)	16.0 ± 0.1	16.1 ± 0.1	16.3 ± 0.1	—
Mean cell hemoglobin concentration (g/dL)	34.5 ± 0.2	34.5 ± 0.2	34.9 ± 0.2	—
Platelets (10^3/µL)	864.4 ± 35.8	896.2 ± 38.0	895.2 ± 60.7	—
Leukocytes (10^3/µL)	4.14 ± 0.42	3.31 ± 0.47	4.82 ± 0.65	—
Segmented neutrophils (10^3/µL)	0.68 ± 0.15	0.50 ± 0.13	0.80 ± 0.14[c]	—
Lymphocytes (10^3/µL)	3.46 ± 0.30	2.80 ± 0.36	3.39 ± 0.29[c]	—
Monocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[c]	—
Eosinophils (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[c]	—

** Significantly different ($P \leq 0.01$) from the vehicle control group by Dunn's or Shirley's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] All animals died before the end of the study.
[c] n=9

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations and Estrous Cycle Characterization for Rats in the 14-Week Feed Study of Theophylline[a]

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Male				
n	10	10	10	10
Weights (g)				
Necropsy body wt	351 ± 8	368 ± 6	364 ± 4	344 ± 6
R. Cauda epididymis	0.202 ± 0.006	0.205 ± 0.007	0.213 ± 0.004	0.185 ± 0.005
R. Epididymis	0.412 ± 0.011	0.428 ± 0.007	0.441 ± 0.005*	0.417 ± 0.007
R. Testis	1.440 ± 0.046	1.484 ± 0.025	1.491 ± 0.018	1.441 ± 0.029
Epididymal spermatozoal measurements				
Sperm motility (%)	77.61 ± 0.55	78.16 ± 0.41	77.70 ± 0.82	77.48 ± 0.77
Abnormal sperm (%)	0.84 ± 0.09	0.96 ± 0.11	1.16 ± 0.21	1.32 ± 0.16
Concentration (10^6/g cauda epididymal tissue)	438 ± 23	395 ± 20	400 ± 21	450 ± 18
Female				
n	10	10	10	10
Weights (g)				
Necropsy body wt	207 ± 3	222 ± 3	206 ± 5	202 ± 8
R. Ovary	0.055 ± 0.006	0.053 ± 0.002	0.064 ± 0.009	0.046 ± 0.003
Uterus	0.509 ± 0.037	0.589 ± 0.067	0.584 ± 0.045	0.449 ± 0.061
Estrous cycle length (days)	4.67 ± 0.29[b]	4.67 ± 0.29[b]	5.13 ± 0.30[c]	5.22 ± 0.28[b]
Estrous stages (% of cycle)				
Diestrus	35.7	34.3	34.3	37.1
Proestrus	18.6	15.7	12.9	15.7
Estrus	25.7	27.1	30.0	31.4
Metestrus	20.0	15.7	22.9	15.7
Uncertain diagnoses	0.0	7.1	0.0	0.0

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

[a] Weights, epididymal spermatozoal parameters, and estrous cycle length are presented as mean ± standard error. Differences from the control group were not significant by Dunnett's test (necropsy body weights, right cauda weights, right testis weights, and female organ weights) or Dunn's test (epididymal spermatozoal measurements and estrous cycle lengths). By multivariate analysis of variance, exposed females did not differ significantly from the control females in the relative length of time spent in the estrous stages.

[b] n=9; estrous cycle was longer than 7 days or unclear in 1 of 10 animals.

[c] n=8; estrous cycle was longer than 7 days or unclear in 2 of 10 animals.

TABLE H2
Summary of Reproductive Tissue Evaluations and Estrous Cycle Characterization for Rats in the 14-Week Gavage Study of Theophylline[a]

	Vehicle Control	37.5 mg/kg	75 mg/kg	150 mg/kg
Male				
n	10	10	10	9
Weights (g)				
Necropsy body wt	336 ± 5	334 ± 5	329 ± 5	321 ± 5
R. Cauda epididymis	0.216 ± 0.012	0.225 ± 0.005	0.216 ± 0.004	0.199 ± 0.004
R. Epididymis	0.442 ± 0.013	0.451 ± 0.009	0.447 ± 0.006	0.421 ± 0.005
R. Testis	1.471 ± 0.020	1.553 ± 0.039	1.496 ± 0.011	1.429 ± 0.027
Epididymal spermatozoal measurements				
Sperm motility (%)	78.67 ± 0.97	80.26 ± 1.08	81.53 ± 1.96	79.58 ± 0.94
Abnormal sperm (%)	0.72 ± 0.12	0.96 ± 0.18	0.82 ± 0.11	0.80 ± 0.12
Concentration (10^6/g cauda epididymal tissue)	394 ± 20	382 ± 13	359 ± 16	413 ± 21
Female				
n	10	10	10	10
Weights (g)				
Necropsy body wt	203 ± 2	198 ± 2	209 ± 3	216 ± 3**[b]
R. Ovary	0.046 ± 0.004	0.062 ± 0.006	0.057 ± 0.004	0.049 ± 0.006[b]
Uterus	0.595 ± 0.072	0.525 ± 0.044	0.587 ± 0.067	0.416 ± 0.034[b]
Estrous cycle length (days)	4.40 ± 0.16	4.89 ± 0.20[c]	4.56 ± 0.29[c]	4.86 ± 0.14[d]
Estrous stages[e] (% of cycle)				
Diestrus	30.0	21.4	35.7	35.7
Proestrus	14.3	25.7	14.3	11.4
Estrus	34.3	35.7	25.7	24.3
Metestrus	21.4	15.7	24.3	27.1
Uncertain diagnoses	0.0	1.4	0.0	1.4

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

[a] Weights, epididymal spermatozoal parameters, and estrous cycle length are presented as mean ± standard error. Differences from the vehicle control group were not significant by Dunnett's test (male necropsy body weights and male and female organ weights) or Dunn's test (epididymal spermatozoal measurements and estrous cycle lengths).

[b] n=9

[c] n=9; estrous cycle was longer than 7 days or unclear in 1 of 10 animals.

[d] n=7; estrous cycle was longer than 7 days or unclear in 3 of 10 animals.

[e] Evidence shows that females exposed to 37.5 or 150 mg/kg differ significantly (Wilk's Criterion, $P \leq 0.05$) from the vehicle control females in the relative length of time spent in the estrous stages. Females in the 37.5 mg/kg group spent more time in proestrus and less time in diestrus and metestrus than vehicle control females. Females in the 150 mg/kg group spent more time in diestrus and metestrus and less time in estrus than the vehicle control females.

TABLE H3
Summary of Reproductive Tissue Evaluations and Estrous Cycle Characterization for Mice in the 14-Week Feed Study of Theophylline[a]

	0 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Male				
n	10	10	10	10
Weights (g)				
Necropsy body wt	34.4 ± 1.0	31.4 ± 0.4**	29.7 ± 0.5**	29.5 ± 0.3**
R. Cauda epididymis	0.019 ± 0.001	0.022 ± 0.001	0.022 ± 0.001*	0.020 ± 0.001
R. Epididymis	0.045 ± 0.001	0.048 ± 0.001	0.050 ± 0.001*	0.049 ± 0.002
R. Testis	0.116 ± 0.001	0.121 ± 0.001	0.116 ± 0.003	0.115 ± 0.001
Epididymal spermatozoal measurements				
Sperm motility (%)	76.34 ± 0.71	75.81 ± 0.57	76.63 ± 0.75	76.37 ± 0.75
Abnormal sperm (%)	1.18 ± 0.18	1.28 ± 0.20	1.08 ± 0.14	1.46 ± 0.29
Concentration (10^6/g cauda epididymal tissue)	876 ± 39	798 ± 23	776 ± 42	817 ± 44
Female				
n	10	10	10	10
Weights (g)				
Necropsy body wt	30.0 ± 0.6	27.8 ± 0.6*	28.0 ± 0.4*	27.9 ± 0.3*
R. Ovary	0.013 ± 0.001	0.012 ± 0.001[b]	0.013 ± 0.001	0.013 ± 0.001
Uterus	0.161 ± 0.010	0.157 ± 0.011	0.143 ± 0.010	0.153 ± 0.013
Estrous cycle length (days)	4.38 ± 0.18[c]	4.78 ± 0.22[d]	4.11 ± 0.26[d]	4.44 ± 0.18[d]
Estrous stages (% of cycle)				
Diestrus	30.0	24.3	28.6	24.3
Proestrus	18.6	22.9	20.0	21.4
Estrus	28.6	32.9	30.0	32.9
Metestrus	22.9	20.0	21.4	21.4

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** ($P \leq 0.01$)
[a] Weights, epididymal spermatozoal parameters, and estrous cycle length are presented as mean ± standard error. Differences from the control group were not significant by Dunnett's test (right testis weights and female organ weights) or Dunn's test (epididymal spermatozoal measurements and estrous cycle lengths). By multivariate analysis of variance, exposed females did not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] n=9
[c] n=8; estrous cycle was longer than 7 days or unclear in 2 of 10 animals.
[d] n=9; estrous cycle was longer than 7 days or unclear in 1 of 10 animals.

TABLE H4
Summary of Reproductive Tissue Evaluations and Estrous Cycle Characterization for Mice in the 14-Week Gavage Study of Theophylline[a]

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Male				
n	10	9	10	7
Weights (g)				
Necropsy body wt	37.9 ± 1.4	35.6 ± 1.2	33.6 ± 0.6**	33.1 ± 0.8**
R. Cauda epididymis	0.019 ± 0.001	0.018 ± 0.001	0.020 ± 0.001	0.016 ± 0.001
R. Epididymis	0.044 ± 0.001	0.042 ± 0.001	0.045 ± 0.001	0.042 ± 0.001
R. Testis	0.119 ± 0.001	0.113 ± 0.002*	0.117 ± 0.002	0.110 ± 0.002**
Epididymal spermatozoal measurements				
Sperm motility (%)	81.82 ± 0.57	81.02 ± 1.27	81.03 ± 0.59	81.30 ± 0.48
Abnormal sperm (%)	0.88 ± 0.14	1.29 ± 0.20	1.02 ± 0.12	1.40 ± 0.20
Concentration (10^6/g cauda epididymal tissue)	888 ± 33	934 ± 60	849 ± 35	1038 ± 74
Female				
n	9	10	10	1
Weights (g)				
Necropsy body wt (g)	30.4 ± 0.6	27.9 ± 0.4*	29.0 ± 0.8	—[b]
R. Ovary	0.013 ± 0.001	0.012 ± 0.001[c]	0.012 ± 0.001	—
Uterus	0.161 ± 0.015	0.148 ± 0.009	0.151 ± 0.012	—
Estrous cycle length (days)	4.44 ± 0.18	4.80 ± 0.20	4.70 ± 0.21	—[d]
Estrous stages[e] (% of cycle)				
Diestrus	20.6	20.0	20.0	0.0
Proestrus	23.8	22.9	20.0	0.0
Estrus	34.9	37.1	38.6	57.1
Metestrus	20.6	20.0	21.4	42.9

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' (male necropsy body weights) or Dunnett's (right testis weight) test
[a] Weights, epididymal spermatozoal parameters, and estrous cycle length are presented as mean ± standard error. Differences from the vehicle control group were not significant by Dunnett's test (right cauda weight, right epididymis weight, female organ weights) or Dunn's test (epididymal spermatozoal measurements and estrous cycle lengths).
[b] One female survived to the end of dosing but died prior to necropsy. Necropsy body weights and organ weights are not available for this animal.
[c] n=9
[d] Estrous cycle was longer than 7 days or unclear in the surviving mouse in this dose group.
[e] Evidence shows that the surviving female exposed to 300 mg/kg differed significantly (Wilk's Criterion, $P \leq 0.05$) from vehicle control females in the relative length of time spent in the estrous stages. This female spent more time in estrus and metestrus and less time in diestrus and proestrus than vehicle control females.

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF THEOPHYLLINE

Theophylline was obtained from Henley and Company, Inc. (New York, NY), in one lot (484), which was used during the 16-day, 14-week, and 2-year feed and gavage studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). Reports on analyses performed in support of the theophylline studies are on file at the National Institute of Environmental Health Sciences.

Before testing was performed, the particle size was reduced by milling the bulk chemical with a Fitzmill®. The chemical, a white powdered solid, was identified as theophylline by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with the literature spectra (*Sadtler Standard Spectra*) of theophylline; the infrared spectrum exhibited the same maxima as a concomitantly analyzed theophylline United States Pharmacopeia (USP) XX reference standard. The infrared and nuclear magnetic resonance spectra are presented in Figures I1 and I2. The melting point was also consistent with a literature reference (*Handbook of Chemistry and Physics*, 1976) and with the USP reference standard.

The purity of lot 484 was determined by elemental analyses, Karl Fischer water analysis, nonaqueous titration, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and USP XX analyses (reaction with a base, acidity, weight loss on drying, residue on ignition, and USP titrimetric assay). For nonaqueous titration as a weak acid, theophylline was dissolved in dimethylformamide and titrated with 0.1 N tetrabutylammonium hydroxide in 2-propanol. The titration was monitored potentiometrically by a calomel reference electrode filled with tetrabutylammonium chloride and by a glass indicating electrode. For nonaqueous amine titration, theophylline was dissolved in a mixture of toluene, acetic anhydride, and glacial acetic acid (18:4:1) and titrated with 0.1 N perchloric acid in glacial acetic acid. The titration was monitored potentiometrically using a combination pH/mV electrode. TLC was performed on Silica Gel 60 F-254 plates with two solvent systems: 1) acetone:chloroform:n-butanol: 25% ammonium hydroxide (30:30:40:10) and 2) carbon tetrachloride:chloroform:methanol (55:35:10). Plates were examined under ultraviolet (254 nm) light and with a spray of ferric chloride and iodine in a mixture of acetone:20% aqueous tartaric acid. Diphenylamine was used as the reference standard. HPLC was performed with a Waters μBondapak C_{18} column using ultraviolet (280 nm) light detection and a system of two solvents: A) tetrabutylammonium hydroxide in water adjusted with 1% phosphoric acid to pH 7.2 and B) tetrabutylammonium hydroxide in methanol with 1% phosphoric acid (90%A:10%B). The flow rate was 1.0 mL/minute.

Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for theophylline. Karl Fischer water analysis indicated 0.052% ± 0.007% water. Nonaqueous titration as a weak acid indicated a purity of 99.3% ± 0.4%, and nonaqueous amine titration indicated a purity of 101.1% ± 0.7%. TLC by each system indicated one major spot and no impurities, identical to results of concomitant analysis of the theophylline USP reference standard. HPLC indicated one major peak and no impurities with areas greater than 0.1% relative to the major peak area. Major peak comparisons of lot 484 with the theophylline USP standard indicated a relative purity of 99.8% ± 1.0%. The USP analyses for theophylline indicated the following results: a clear solution was produced when the sample was reacted with a 1 N potassium hydroxide solution; titration of the acidic components required less than 1 mL of 0.02 N sodium hydroxide; weight loss on drying was 0.090% ± 0.004%; and residue on ignition was 0.048% ± 0.003%. The USP titrimetric assay indicated a purity of 100.0% ± 0.7%, consistent with the

USP purity requirements of 98.5% to 101.0% for theophylline. The overall purity was determined to be greater than 99%.

Accelerated stability studies of the bulk chemical were performed by the analytical chemistry laboratory. Theophylline was stored in amber septum vials with Teflon®-lined septa for 2 weeks at temperatures of -20°, 5°, 25°, and 60° C. Samples were analyzed by HPLC by the system described for the purity analyses but with a solvent ratio of 80A:20B and with caffeine added as an internal standard. These studies indicated that theophylline was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature in a plastic bag in the original metal container or in amber glass bottles. Stability was monitored by the study laboratory during the 16-day gavage studies and all 14-week studies by HPLC and nonaqueous titration as a weak acid and during the 2-year studies by HPLC. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Feed Studies

The dose formulations were prepared twice during the 16-day studies and weekly during the 14-week studies by mixing theophylline with feed (Table I1). Premixes of theophylline and feed were prepared by hand and then the premixes were blended with additional feed in a Patterson-Kelly twin-shell blender for 15 minutes, using an intensifier bar for the initial 5 minutes. Formulations were stored in double-thickness plastic bags (during the 16-day studies, bags were stored inside a second opaque plastic bag) inside labeled Bain-Marie containers at 5° C for up to 14 days during the 16-day studies and for up to 15 days during the 14-week studies.

Homogeneity studies of the 1,000 and 4,000 ppm dose formulations were performed by the study laboratory using HPLC. The analytical chemistry laboratory conducted homogeneity studies using ultraviolet/visible spectroscopy and stability studies using HPLC on 1,000 and 10,000 ppm formulations. Homogeneity was confirmed. The stability of the dose formulations was confirmed for 21 days at -20° C, and was confirmed for 7 days at room temperature when exposed to air and light. Losses of 3% and 6%, respectively, were noted when dosed feed was stored at 5° C and at room temperature for 21 days.

Periodic analyses of the dose formulations of theophylline were conducted at the study laboratory using HPLC. HPLC was performed with a Waters μBondapak C_{18} column using ultraviolet (280 nm) light detection and a system of two solvents: A) 0.005 M tetrabutylammonium phosphate in water adjusted with 1% phosphoric acid to pH 7.2 and B) 0.005 M tetrabutylammonium phosphate in methanol with 1% phosphoric acid (80%A:20%B). The flow rate was 1.0 mL/minute. During the 16-day studies, dose formulations were analyzed once (Table I2). For the 14-week studies, the initial, middle, and final dose preparations were analyzed (Table I3). All dose formulations analyzed and used during the 16-day (10/10) and 14-week (9/9) feed studies were within 10% of the target concentration, with no value greater than 105% (16-day studies) or 104% (14-week studies) of the target concentration. Results of periodic referee analyses performed by the analytical chemistry laboratory agreed with the results for the 14-week studies obtained by the study laboratory (Table I4).

Gavage Studies

The dose formulations were prepared twice during the 16-day studies, weekly during the 14 week studies, and every 2 weeks during the 2-year studies. Dose formulations were prepared by mixing theophylline with corn oil to give the required concentrations (Table I1). The dose formulations were stored at room temperature in amber glass bottles for up to 15 days during the 16-day and 14-week studies and for up to 20 days during the 2-year studies.

Homogeneity studies of the 1.36 and 87.1 mg/g dose preparations used during the 16-day studies and the 0.82 and 16.3 mg/g dose preparations used during the 2-year studies were performed by the study laboratory using ultraviolet/visible spectroscopy (250 to 290 nm). The analytical chemistry laboratory also performed homogeneity testing on a 100.1 mg/mL suspension using ultraviolet/visible spectroscopy (270 nm). A stability study was also conducted at the analytical chemistry laboratory on a 1 mg/mL (1.1 mg/g) suspension of theophylline in corn oil using HPLC. HPLC was performed with a Brownlee MPLC® RP-18 column using ultraviolet (280 nm) light detection and a system of water and methanol (80:20). The flow rate was 1.3 mL/minute. Homogeneity was confirmed. The stability of the dose formulations was confirmed for at least 21 days at 5° C and at room temperature when stored in sealed vessels and protected from light and was confirmed for 3 hours when stored exposed to air and light.

Periodic analyses of the dose formulations of theophylline were conducted at the study laboratory using ultraviolet/visible spectroscopy (16-day and 14-week studies) or by visible spectroscopy (2-year studies). During the 16-day studies, dose preparations were analyzed once (Table I5). For the 14-week studies, dose preparations from the beginning, middle, and end of the studies were analyzed (Table I6). During the 2-year studies, dose preparations were analyzed approximately every 6 to 10 weeks (Table I7). All dose formulations analyzed and used during the 16-day (12/12), 14-week (18/18), and 2-year (81/81) studies were within 10% of the target concentration, with no value greater than 105% (16-day studies), 104% (14-week studies), or 107% (2-year studies) of the target concentration. In addition to dose formulation analysis prior to dosing, samples collected after dosing (animal room samples) were analyzed periodically. All animal room samples from formulations used during the 16-day (12/12) and 14-week (18/18) studies were within 10% of target concentration. For the 2-year studies, 84% (26/31) were within 10% of the target concentration. The remaining five samples ranged from 28% to 112% of the target concentration. Periodic analyses of the corn oil vehicle by the study laboratory demonstrated that peroxide levels were within the acceptable limit of 10 mEq/kg designated for the 16-day and 14-week studies. For the 2-year studies, the maximum acceptable limit for peroxide was 3 mEq/kg, and all samples were below this level with the exception of two lots. One lot that was slightly above the acceptable peroxide level was used for dosing until another lot of corn oil could be obtained. Results of periodic referee analyses performed by the analytical chemistry laboratory during the 14-week studies agreed with the results obtained by the study laboratory (Table I8).

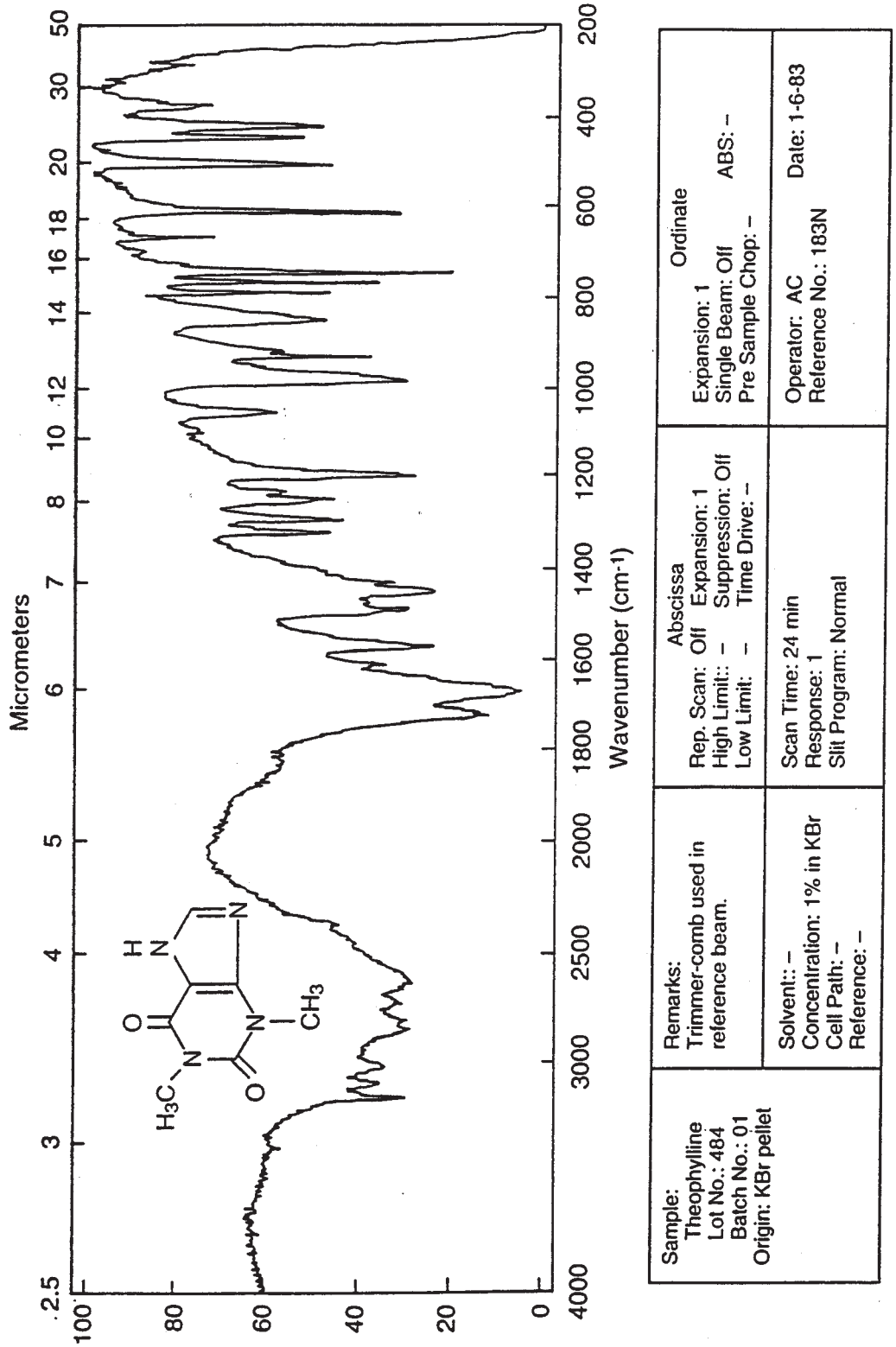

FIGURE I1
Infrared Absorption Spectrum of Theophylline

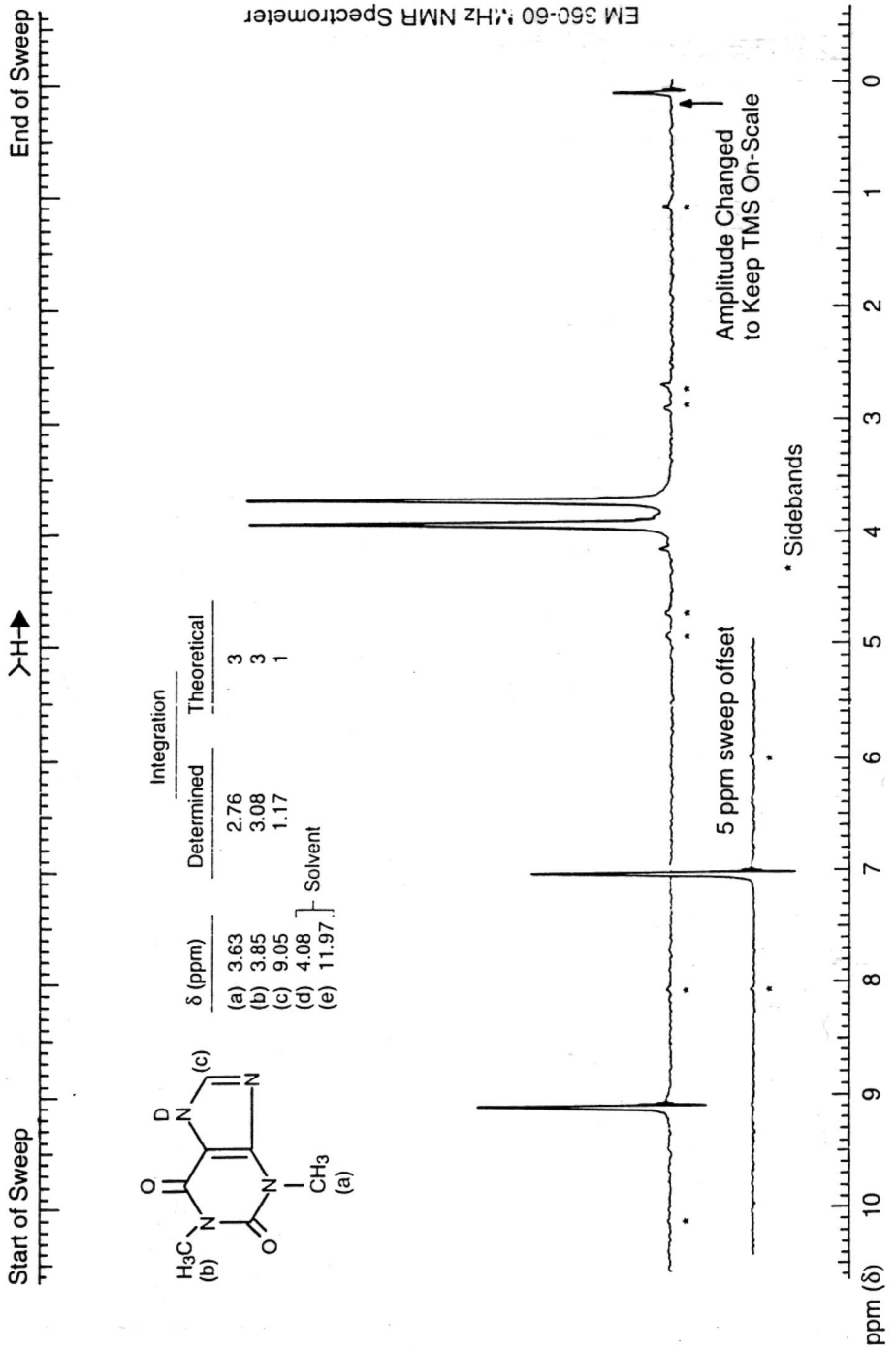

FIGURE I2
Nuclear Magnetic Resonance Spectrum of Theophylline

TABLE I1
Preparation and Storage of Dose Formulations in the Feed and Gavage Studies of Theophylline

16-Day Feed Studies	14-Week Feed Studies
Preparation A premix of feed and theophylline was prepared, then layered into the remaining feed and blended in a Patterson-Kelly twin-shell blender with the intensifier bar on for 5 minutes and off for 10 minutes. Doses were prepared twice.	Same as 16-day feed studies; doses were prepared weekly.
Chemical Lot Number 484	484
Maximum Storage Time 14 days	15 days
Storage Conditions Stored in double-thickness plastic bags within a second opaque plastic bag and placed inside labeled Bain-Marie containers at 5° C	Stored in double-thickness plastic bags inside labeled Bain-Marie containers at 5° C
Study Laboratory Southern Research Institute (Birmingham, AL)	Same as 16-day feed studies
Referee Laboratory None	Midwest Research Institute (Kansas City, MO)

TABLE I1
Preparation and Storage of Dose Formulations in the Feed and Gavage Studies of Theophylline (continued)

16-Day Gavage Studies	14-Week Gavage Studies	2-Year Gavage Studies
Preparation		
Theophylline and corn oil were mixed in a beaker or aspirator bottle and stirred with a magnetic stirbar for 3 minutes. Corn oil was added to bring the mixture to specified volume and blended for 3 minutes. The suspension was degassed and restirred. Doses were prepared twice.	Same as 16-day gavage studies; doses were prepared weekly.	Same as 16-day gavage studies; doses were prepared every 2 weeks.
Chemical Lot Number		
484	484	484
Maximum Storage Time		
15 days	15 days	20 days
Storage Conditions		
Stored in amber glass bottles at room temperature	Same as 16-day gavage studies	Same as 16-day gavage studies
Study Laboratory		
Southern Research Institute (Birmingham, AL)	Same as 16-day gavage studies	Same as 16-day gavage studies
Referee Laboratory		
None	Midwest Research Institute (Kansas City, MO)	None

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 16-Day Feed Studies of Theophylline

Date Prepared	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats			
21 November 1983	500	526	+5
	1,000	1,040	+4
	2,000	2,020	+1
	4,000	4,000	0
	8,000	8,140	+2
Mice			
14 November 1983	500	472	−6
	1,000	1,000	0
	2,000	2,010	+1
	4,000	4,080	+2
	8,000	8,140	+2

[a] Results of duplicate analyses

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of Theophylline

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
5 February 1986[b]	6 February 1986	1,000	1,010	+1
		1,000	1,020	+2
		1,000	968	−3
		4,000	4,020	+1
		4,000	4,000	0
		4,000	4,020	+1
19 March 1986	19 March 1986	1,000	1,040	+4
		2,000	2,010	+1
		4,000	4,020	+1
7 May 1986	7-8 May 1986	1,000	1,040	+4
		2,000	2,040	+2
		4,000	4,030	+1
25 June 1986	25-26 June 1986	1,000	985	−2
		2,000	1,990	−1
		4,000	3,960	−1

[a] Results of duplicate analyses
[b] Homogeneity analyses, not used for dosing. For each target concentration, the three results given are the concentrations determined from samples collected from the top right, top left, and bottom of the formulation and are reported in that order.

TABLE I4
Results of Referee Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of Theophylline

Date Prepared	Target Concentration (ppm)	Determined Concentration	
		Study Laboratory[a] (ppm)	Referee Laboratory[b] (ppm)
19 March 1986	1,000	1,040	978 ± 2
25 June 1986	2,000	1,990	1,949 ± 13

[a] Results of duplicate analyses
[b] Results of triplicate analyses (mean ± standard error)

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 16-Day Gavage Studies of Theophylline

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration[a] (mg/g)	Difference from Target (%)
21 May 1985[b]	22 May 1985	1.36	1.46	+7
		1.36	1.54	+13
		1.36	1.48	+9
		87.1	88.6	+2
		87.1	89.2	+2
		87.1	90.7	+4
Rats				
4 June 1985	4-5 June 1985	2.72	2.78	+2
		5.44	5.57	+2
		10.9	10.9	0
		21.8	22.2	+2
		43.5	44.4	+2
		87.1	85.9	−1
	25-26 June 1985[c]	2.72	2.83	+4
		5.44	5.59	+3
		10.9	11.1	+2
		21.8	22.1	+1
		43.5	43.9	+1
		87.1	86.4	−1
Mice				
28 May 1985	29-30 May 1985	1.36	1.43	+5
		2.72	2.66	−2
		5.44	5.60	+3
		10.9	11.0	+1
		21.8	21.8	0
		43.5	43.4	0
	19-20 June 1985[c]	1.36	1.36	0
		2.72	2.68	−1
		5.44	5.56	+2
		10.9	11.0	+1
		21.8	21.9	0
		43.5	43.0	−1

[a] Results of duplicate analyses. Rat dosing volume = 5 mL/kg; 2.72 mg/g = 12.5 mg/kg, 5.44 mg/g = 25 mg/kg, 10.9 mg/g = 50 mg/kg, 21.8 = 100 mg/kg, 43.5 mg/g = 200 mg/kg, 87.1 mg/g = 400 mg/kg. Mouse dosing volume = 10 mL/kg; 1.36 mg/g = 12.5 mg/kg, 2.72 mg/g = 25 mg/kg, 5.44 mg/g = 50 mg/kg, 10.9 mg/g = 100 mg/kg, 21.8 mg/g = 200 mg/kg, 43.5 mg/g = 400 mg/kg.

[b] Homogeneity analyses, not used for dosing. For each target concentration, the three results given are the concentrations determined from samples collected from the top, middle, and bottom of the beaker and are reported in that order.

[c] Animal room samples

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Theophylline

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration[a] (mg/g)	Difference from Target (%)
Rats				
9 April 1986	9-10 April 1986	8.09	8.28	+2
		8.09	8.34	+3
		16.1	16.2	+1
		16.1	16.2	+1
		31.6	32.2	+2
		31.6	31.8	+1
	24 April 1986[b]	8.09	8.24	+2
		8.09	8.43	+4
		16.1	16.4	+2
		16.1	16.6	+3
		31.6	32.3	+2
		31.6	31.8	+1
21 May 1986	21-22 May 1986	8.09	8.26	+2
		8.09	8.39	+4
		16.1	16.7	+4
		16.1	16.5	+2
		31.6	32.4	+3
		31.6	32.1	+2
	3-4 June 1986[b]	8.09	8.23	+2
		8.09	8.40	+4
		16.1	16.7	+4
		16.1	16.6	+3
		31.6	32.1	+2
		31.6	31.5	0
2 July 1986	7 July 1986	8.09	8.36	+3
		16.1	16.6	+3
		31.6	32.2	+2
	17-18 July 1986[b]	8.09	8.18	+1
		16.1	16.1	0
		31.6	31.2	−1
Mice				
9 April 1986	9-10 April 1986	8.09	8.28	+2
		8.09	8.34	+3
		16.1	16.2	+1
		16.1	16.2	+1
		31.6	32.2	+2
		31.6	31.8	+1
	24 April 1986[b]	8.09	8.24	+2
		8.09	8.43	+4
		16.1	16.4	+2
		16.1	16.6	+3
		31.6	32.3	+2
		31.6	31.8	+1

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Theophylline (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice (continued)				
21 May 1986	21-22 May 1986	8.09	8.26	+2
		8.09	8.39	+4
		16.1	16.7	+4
		16.1	16.5	+2
		31.6	32.4	+3
		31.6	32.1	+2
	3-4 June 1986[b]	8.09	8.23	+2
		8.09	8.40	+4
		16.1	16.7	+4
		16.1	16.6	+3
		31.6	32.1	+2
		31.6	31.5	0
2 July 1986	7 July 1986	8.09	8.36	+3
		16.1	16.6	+3
		31.6	32.2	+2
	17-18 July 1986[b]	8.09	8.18	+1
		16.1	16.1	0
		31.6	31.2	−1
9 July 1986	11 July 1986	8.09	8.36	+3
		16.1	16.3	+1
		31.6	32.6	+3
	24-25 July 1986[b]	8.09	8.66	+7
		16.1	16.6	+3
		31.6	32.4	+3

[a] Results of duplicate analyses. Rat dosing volume = 5 mL/kg; 8.09 mg/g = 37.5 mg/kg, 16.1 mg/g = 75 mg/kg, 31.6 mg/g = 150 mg/kg. Mouse dosing volume = 10 mL/kg; 8.09 mg/g = 75 mg/kg, 16.1 mg/g = 150 mg/kg, 31.6 mg/g = 300 mg/kg.
[b] Animal room samples

TABLE I7
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Theophylline

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration[a] (mg/g)	Difference from Target (%)
12 September 1990[b]	12-14 September 1990	0.82	0.833	+2
		0.82	0.856	+4
		0.82	0.833	+2
		16.3	16.8	+3
		16.3	16.6	+2
		16.3	16.7	+2
Rats				
10 October 1990	11 October 1990	1.63	1.64	+1
		5.44	5.46	0
		16.3	16.4	+1
	30-31 October 1990[c]	1.63	1.67	+3
		5.44	5.49	+1
		16.3	16.3	+1
5 December 1990	6-7 December 1990	1.63	1.61	−1
		5.44	5.48	+1
		16.3	16.3	0
30 January 1991	31 January 1991	1.63	1.52	−6
		5.44	5.55	+2
		16.3	16.6	+2
27 March 1991	28 March 1991	1.63	1.64	+1
		5.44	5.58	+3
		16.3	16.9	+4
	17-18 April 1991[c]	1.63	1.60	−2
		5.44	5.39	−1
		16.3	16.1	−1
22 May 1991	22-23 May 1991	1.63	1.66	+2
		5.44	5.46	0
		16.3	16.5	+1
17 July 1991	17-18 July 1991	1.63	1.66	+2
		5.44	5.66	+4
		16.3	16.7	+2
11 September 1991	11-12 September 1991	1.63	1.60	−2
		5.44	5.42	0
		16.3	16.6	+2
	2-3 October 1991[c]	1.63	1.82	+12
		5.44	3.63	−33
		16.3	15.3	−6
		16.3	16.9	+4
6 November 1991[d]	6-7 November 1991	1.63	1.63	0
		5.44	5.35	−2
		16.3	16.6	+2

TABLE I7
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Theophylline (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
11 November 1991	11-12 November 1991	1.63	1.63	0
		5.44	5.75	+6
		16.3	17.2	+6
15 January 1992	15-16 January 1992	1.63	1.75	+7
		5.44	5.64	+4
		16.3	16.7	+2
	27 January 1992[c]	1.63	1.66	+2
		5.44	5.45	0
		16.3	16.5	+1
11 March 1992	12 March 1992	1.63	1.62	−1
		5.44	5.45	0
		16.3	16.6	+2
	1-2 April 1992[c]	1.63	1.61	−1
		5.44	5.55	+2
		16.3	16.6	+2
6 May 1992	6-7 May 1992	1.63	1.68	+3
		5.44	5.61	+3
		16.3	16.4	+1
15 July 1992	15-16 July 1992	1.63	1.66	+2
		5.44	5.55	+2
		16.3	16.5	+1
23 September 1992	23-24 September 1992	1.63	1.75	+7
		5.44	5.54	+2
		16.3	16.5	+1
	14 October 1992[c]	1.63	1.51	−7
		5.44	5.13	−6
		16.3	16.3	0
7 October 1992	21 October 1992	1.63	1.70	+4
		5.44	5.19	−5
		16.3	16.2	−1
Mice				
10 October 1990	11 October 1990	0.816	0.830	+1
		1.63	1.64	+1
		2.72	2.78	+2
		5.44	5.46	0
		8.16	8.22	+1
		16.3	16.4	+1

TABLE I7
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Theophylline (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice (continued)				
	30-31 October 1990[c]	0.816	0.228	−72
		1.63	1.67	+3
		2.72	2.82	+4
		5.44	5.49	+1
		8.16	7.90	−3
		16.3	16.3	0
5 December 1990	6-7 December 1990	0.816	0.748	−9
		1.63	1.61	−1
		2.72	2.84	+4
		5.44	5.48	+1
		8.16	8.32	+2
		16.3	16.3	0
30 January 1991	31 January 1991	0.816	0.766	−6
		1.63	1.52	−6
		2.72	2.80	+3
		5.44	5.55	+2
		8.16	8.41	+3
		16.3	16.6	+2
27 March 1991	28 March 1991	0.816	0.852	+4
		1.63	1.64	+1
		2.72	2.77	+2
		5.44	5.58	+3
		8.16	8.38	+3
		16.3	16.9	+4
	17-18 April 1991[c]	0.816	0.608	−25
		1.63	1.60	−2
		2.72	2.80	+3
		5.44	5.39	−1
		8.16	7.98	−2
		16.3	16.1	−1
22 May 1991	22-23 May 1991	0.816	0.822	+1
		1.63	1.66	+2
		2.72	2.69	−1
		5.44	5.46	0
		8.16	8.31	+2
		16.3	16.5	+1
17 July 1991	17-18 July 1991	0.816	0.863	+6
		1.63	1.66	+2
		2.72	2.80	+3
		5.44	5.66	+4
		8.16	8.41	+3
		16.3	16.7	+2

TABLE I7
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Theophylline (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice (continued)				
11 September 1991	11-12 September 1991	0.816	0.795	−3
		1.63	1.60	−2
		2.72	2.68	−1
		5.44	5.42	0
		8.16	8.44	+3
		16.3	16.6	+2
	2-3 October 1991[c]	0.816	0.576	−29
		1.63	1.82	+12
		2.72	2.70	−1
		5.44	3.63	−33
		8.16	7.93	−3
		16.3	15.3	−6
		16.3	16.9	+4
6 November 1991[d]	6-7 November 1991	0.816	0.804	−1
		1.63	1.63	0
		2.72	2.68	−1
		5.44	5.35	−2
		8.16	8.29	+2
		16.3	16.6	+2
11 November 1991	11-12 November 1991	0.816	0.809	−1
		1.63	1.63	0
		2.72	2.71	0
		5.44	5.75	+6
		8.16	8.70	+7
		16.3	17.2	+6
15 January 1992	15-16 January 1992	0.816	0.829	+2
		1.63	1.75	+7
		2.72	2.76	+1
		5.44	5.64	+4
		8.16	8.40	+3
		16.3	16.7	+2
	27 January 1992[c]	1.63	1.66	+2
		5.44	5.45	0
		16.3	16.5	+1
11 March 1992	12 March 1992	0.816	0.801	−2
		1.63	1.62	−1
		2.72	2.74	+1
		5.44	5.45	0
		8.16	8.21	+1
		16.3	16.6	+2

TABLE I7
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Theophylline (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice (continued)				
	1-2 April 1992[c]	0.816	0.841	+3
		1.63	1.61	−1
		2.72	2.75	+1
		5.44	5.55	+2
		8.16	8.27	+1
		16.3	16.6	+2
6 May 1992	6-7 May 1992	0.816	0.796	−2
		1.63	1.68	+3
		2.72	2.74	+1
		5.44	5.61	+3
		8.16	8.07	−1
		16.3	16.4	+1
15 July 1992	15-16 July 1992	0.816	0.812	0
		1.63	1.66	+2
		2.72	2.78	+2
		5.44	5.55	+2
		8.16	7.98	−2
		16.3	16.5	+1
23 September 1992	23-24 September 1992	0.816	0.918[e]	+13
		1.63	1.75	+7
		2.72	2.72	0
		5.44	5.54	+2
		8.16	8.20	0
		16.3	16.5	+1
	14 October 1992[c]	1.63	1.51	−7
		5.44	5.13	−6
		16.3	16.3	0
25 September 1992	25 September 1992	0.816	0.837[f]	+3

[a] Results of duplicate analyses. Rat dosing volume = 5 mL/kg; 1.63 mg/g = 7.5 mg/kg, 5.44 mg/g = 25 mg/kg, 16.3 mg/g = 75 mg/kg. Mouse dosing volume = 10 mL/kg; 0.816 mg/g = 7.5 mg/kg, 1.63 mg/g = 15 mg/kg, 2.72 mg/g = 25 mg/kg, 5.44 mg/g = 50 mg/kg, 8.16 mg/g = 75 mg/kg, 16.3 mg/g = 150 mg/kg.
[b] Homogeneity analyses, not used for dosing. For each target concentration, the three results given are the concentrations determined from samples collected from the top, middle, and bottom of the beaker and are reported in that order.
[c] Animal room samples
[d] The corn oil used for this mix was analyzed prior to dosing and found to be rancid (5.52 mEq/kg peroxide). A remix with a different lot of corn oil was formulated on 11 November 1991.
[e] Not used for dosing
[f] Results of remix

TABLE I8
Results of Referee Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Theophylline

Date Prepared	Target Concentration (mg/g)	Determined Concentration	
		Study Laboratory[a] (mg/g)	Referee Laboratory[b] (mg/g)
9 April 1986	31.6	32.2	30.6 ± 0.7
2 July 1986	16.1	16.6	16.1 ± 0.1

[a] Results of duplicate analyses
[b] Results of triplicate analyses (mean ± standard error)

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

TABLE J1
Ingredients of NIH-07 Rat and Mouse Ration[a]

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

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

TABLE J2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
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 J3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	23.40 ± 0.57	22.2) 24.3	24
Crude fat (% by weight)	5.32 ± 0.19	5.00) 5.90	24
Crude fiber (% by weight)	3.36 ± 0.38	2.60) 4.30	24
Ash (% by weight)	6.45 ± 0.19	6.12) 6.81	24
Amino Acids (% of total diet)			
Arginine	1.280 ± 0.083	1.110) 1.390	11
Cystine	0.308 ± 0.071	0.181) 0.400	11
Glycine	1.158 ± 0.048	1.060) 1.220	11
Histidine	0.584 ± 0.027	0.531) 0.630	11
Isoleucine	0.917 ± 0.033	0.867) 0.965	11
Leucine	1.975 ± 0.051	1.850) 2.040	11
Lysine	1.274 ± 0.049	1.200) 1.370	11
Methionine	0.437 ± 0.109	0.306) 0.699	11
Phenylalanine	0.999 ± 0.120	0.665) 1.110	11
Threonine	0.904 ± 0.058	0.824) 0.985	11
Tryptophan	0.218 ± 0.153	0.107) 0.671	11
Tyrosine	0.685 ± 0.094	0.564) 0.794	11
Valine	1.086 ± 0.055	0.962) 1.170	11
Essential Fatty Acids (% of total diet)			
Linoleic	2.407 ± 0.227	1.830) 2.570	10
Linolenic	0.259 ± 0.065	0.100) 0.320	10
Vitamins			
Vitamin A (IU/kg)	6,768 ± 1,337	5,730) 11,450	24
Vitamin D (IU/kg)	4,450 ± 1,382	3,000) 6,300	4
α-Tocopherol (ppm)	35.43 ± 8.98	22.5) 48.9	11
Thiamine (ppm)	17.38 ± 2.08	14.0) 22.0	24
Riboflavin (ppm)	7.83 ± 0.923	6.10) 9.00	11
Niacin (ppm)	99.22 ± 24.27	65.0) 150.0	11
Pantothenic acid (ppm)	30.55 ± 3.52	23.0) 34.6	11
Pyridoxine (ppm)	9.11 ± 2.53	5.60) 14.0	11
Folic acid (ppm)	2.46 ± 0.63	1.80) 3.70	11
Biotin (ppm)	0.268 ± 0.047	0.190) 0.354	11
Vitamin B_{12} (ppb)	40.5 ± 19.1	10.6) 65.0	11
Choline (ppm)	2,991 ± 382	2,300) 3,430	10
Minerals			
Calcium (%)	1.18 ± 0.10	1.00) 1.49	24
Phosphorus (%)	0.92 ± 0.05	0.76) 1.00	24
Potassium (%)	0.886 ± 0.063	0.772) 0.971	9
Chloride (%)	0.529 ± 0.087	0.380) 0.635	9
Sodium (%)	0.316 ± 0.033	0.258) 0.371	11
Magnesium (%)	0.166 ± 0.010	0.148) 0.181	11
Sulfur (%)	0.272 ± 0.059	0.208) 0.420	10
Iron (ppm)	350.5 ± 87.3	255.0) 523.0	11
Manganese (ppm)	92.48 ± 5.14	81.7) 99.4	11
Zinc (ppm)	59.33 ± 10.2	46.1) 81.6	11
Copper (ppm)	11.81 ± 2.50	8.09) 15.4	11
Iodine (ppm)	3.54 ± 1.19	1.52) 5.83	10
Chromium (ppm)	1.66 ± 0.46	0.85) 2.09	11
Cobalt (ppm)	0.76 ± 0.23	0.49) 1.15	7

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

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.45 ± 0.18	0.10) 0.70	24
Cadmium (ppm)	0.13 ± 0.07	0.04) 0.20	24
Lead (ppm)	0.35 ± 0.25	0.10) 1.00	24
Mercury (ppm)[c]	0.02	0.02) 0.03	24
Selenium (ppm)	0.33 ± 0.11	0.05) 0.40	24
Aflatoxins (ppm)	< 5.0		24
Nitrate nitrogen (ppm)[d]	8.44 ± 4.10	2.90) 17.0	24
Nitrite nitrogen (ppm)[d]	0.14 ± 0.06	0.10) 0.30	24
BHA (ppm)[e]	1.83 ± 1.97	1.00) 10.0	24
BHT(ppm)[e]	1.6 ± 1.61	1.0) 8.00	24
Aerobic plate count (CFU/g)	99,875 ± 164,883	4,100) 712,400	24
Coliform (MPN/g)	3 ± 0.3	3) 4	24
Escherichia coli (MPN/g)	< 3		24
Salmonella (MPN/g)	Negative		24
Total nitrosoamines (ppb)[f]	7.34 ± 1.77	4.70) 11.40	24
N-Nitrosodimethylamine (ppb)[f]	5.39 ± 1.20	2.90) 8.20	24
N-Nitrosopyrrolidine (ppb)[f]	1.95 ± 1.01	1.00) 4.30	24
Pesticides (ppm)			
α-BHC	< 0.01		24
β-BHC	< 0.02		24
γ-BHC	< 0.01		24
δ-BHC	< 0.01		24
Heptachlor	< 0.01		24
Aldrin	< 0.01		24
Heptachlor epoxide	< 0.01		24
DDE	< 0.01		24
DDD	< 0.01		24
DDT	< 0.01		24
HCB	< 0.01		24
Mirex	< 0.01		24
Methoxychlor	< 0.05		24
Dieldrin	< 0.01		24
Endrin	< 0.01		24
Telodrin	< 0.01		24
Chlordane	< 0.05		24
Toxaphene	< 0.10		24
Estimated PCBs	< 0.20		24
Ronnel	< 0.01		24
Ethion	< 0.02		24
Trithion	< 0.05		24
Diazinon	< 0.10		24
Methyl parathion	< 0.02		24
Ethyl parathion	< 0.02		24
Malathion	0.24 ± 0.23	0.05) 0.97	24
Endosulfan I	< 0.01		24
Endosulfan II	< 0.01		24
Endosulfan sulfate	< 0.03		24

[a] CFU = colony-forming units, MPN = most probable number, BHC = hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] All values except for the lots milled September, November, and December 1991 were less than the detection limit. The detection limit is given as the mean.
[d] Sources of contamination: alfalfa, grains, and fish meal
[e] Sources of contamination: soy oil and fish meal
[f] All values were corrected for percent recovery.

APPENDIX K
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from up to five randomly selected male and female rats and mice during the 14-week feed and gavage studies and the 2-year gavage studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to Microbiological Associates, Inc. (Bethesda, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
14-Week Feed Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/ sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	Study termination
KRV (Kilham rat virus)	Study termination
14-Week Gavage Study	
ELISA	
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	Study termination
RCV/SDA	Study termination
Sendai	Study termination
Hemagglutination Inhibition	
H-1	Study termination
KRV	Study termination

Method and Test	Time of Analysis
2-Year Gavage Study	
ELISA	
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	6 and 12 months, study termination
RCV/SDA	6 and 12 months, study termination
Sendai	6 and 12 months, study termination
Hemagglutination Inhibition	
H-1	6 and 12 months, study termination
KRV	6 and 12 months, study termination

MICE

14-Week Feed Study	
Complement Fixation	
LCM (lymphocytic choriomeningitis virus)	Study termination
ELISA	
Ectromelia virus	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
Mouse adenoma virus	Study termination
MHV (mouse hepatitis virus)	Study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
Immunofluorescence Assay	
EDIM (epizootic diarrhea of infant mice)	Study termination
Hemagglutination Inhibition	
K (papovavirus)	Study termination
MVM (minute virus of mice)	Study termination
Polyoma virus	Study termination

Method and Test	Time of Analysis
14-Week Gavage Study	
Complement Fixation	
LCM	Study termination
ELISA	
CARB (cilia-associated respiratory bacillus)	Study termination
Ectromelia virus	Study termination
GDVII	Study termination
Mouse adenoma virus	Study termination
MHV	Study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
Immunofluorescence Assay	
EDIM	Study termination
Hemagglutination Inhibition	
K	Study termination
MVM	Study termination
Polyoma virus	Study termination
2-Year Gavage Study	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM	12 and 18 months, study termination
GDVII	6, 12, and 18 months, study termination
LCM	6, 12, and 18 months, study termination
Mouse adenoma virus-FL	6, 12, and 18 months, study termination
MHV	2, 6, 12, and 18 months, study termination
PVM	6, 12, and 18 months, study termination
Reovirus 3	2, 6, 12, and 18 months, study termination
Sendai	2, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
EDIM	6 months
Mouse adenoma virus-FL	12 months
Reovirus 3	2 months, study termination
Hemagglutination Inhibition	
K	6, 12, and 18 months, study termination
MVM	6, 12, and 18 months, study termination
Polyoma virus	6, 12, and 18 months, study termination

RESULTS

One mouse in the 14-week feed study, two rats in the 14-week gavage study, and one rat in the 2-year study had positive titers to *M. arthritidis* at study termination. Further evaluation of the sera positive for *M. arthritidis* by immunoblot and Western blot procedures indicated that the positive titers may have been due to a cross reaction with antibodies of nonpathogenic *Mycoplasma* or other agents. There were no clinical findings or histopathologic changes indicative of *M. arthritidis* infection in the animals with the positive titers. Accordingly, the *M. arthritidis*-positive titers were considered to be false positives.

One mouse in the 2-year gavage study was positive for Reovirus 3 using an immunofluorescence assay, and the ELISA result for Reovirus 3 was borderline positive for this animal. However, because Western blot analyses were negative for specific antibodies and there was no increased incidence within the colonies, the immunofluorescence assay and ELISA results were interpreted as nonspecific.

APPENDIX L
IMPACT OF *HELICOBACTER HEPATICUS* INFECTION IN B6C3F$_1$ MICE FROM 12 NTP 2-YEAR CARCINOGENESIS STUDIES

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IMPACT OF *HELICOBACTER HEPATICUS* INFECTION IN B6C3F$_1$ MICE FROM 12 NTP 2-YEAR CARCINOGENESIS STUDIES

ABSTRACT

Male and female B6C3F$_1$ mice from 12 NTP 2-year carcinogenesis studies were found to be infected with *Helicobacter hepaticus*. Many of the male mice from nine of these studies ("affected" studies) had an associated hepatitis. The current evaluations were performed in an attempt to determine if the data from the *H. hepaticus*-affected NTP B6C3F$_1$ mouse studies were compromised and unsuitable for cancer hazard identification. The incidences of neoplasms of the liver (both hepatocellular neoplasms and hemangiosarcoma), but not of other organs in control male B6C3F$_1$ mice, were found to be increased in affected studies compared to control males from unaffected studies. The increased incidence of hepatocellular neoplasms was observed in those males exhibiting *H. hepaticus*-associated hepatitis. Other observations further differentiated control male mice from affected and unaffected studies. H-*ras* codon 61 CAA-to-AAA mutations were less common in liver neoplasms in males from affected studies compared to historical and unaffected study controls. In addition, increases in cell proliferation rates and apoptosis were observed in the livers of male mice with *H. hepaticus*-associated hepatitis. These data support the hypothesis that the increased incidence of liver neoplasms is associated with *H. hepaticus* and that hepatitis may be important in the pathogenesis. Therefore, interpretation of carcinogenic effects in the liver of B6C3F$_1$ mice may be confounded if there is *H. hepaticus*-associated hepatitis.

INTRODUCTION

Helicobacter-Induced Diseases

Since the bacterium *H. pylori* was isolated from humans in 1983, numerous *Helicobacter* species have been identified in several laboratory and domestic animal species. Their pathogenicity varies, with some species inducing significant disease while others appear merely to colonize the gastrointestinal tract. *H. pylori* is known to cause chronic gastritis and peptic ulcers in humans (Marshall and Warren, 1984; Graham, 1989; Lee *et al.*, 1993) and, more recently, has been linked to adenocarcinoma and mucosa-associated lymphoma of the stomach (Fox *et al.*, 1989; Nomura *et al.*, 1991; Parsonnet *et al.*, 1991; Wotherspoon *et al.*, 1993). Based on epidemiological and pathology findings, the International Agency for Research on Cancer (1994) has classified *H. pylori* as a group 1 carcinogen in humans. *H. hepaticus* is associated with an increase in liver neoplasm incidences in A/JCr mice (Ward *et al.*, 1994a; Fox *et al.*, 1996).

H. hepaticus commonly colonizes the gastrointestinal tract of many strains of mice from many sources (Fox *et al.*, 1994; Ward *et al.*, 1994b; Shames *et al.*, 1995). It has been shown to be pathogenic, with hepatitis highly prevalent in some strains of mice (A/JCr, BALB/cAnNCr, C3H/HeNCr, SJL/NCr, and SCID/NCr) (Ward *et al.*, 1994b). Intestinal colonization does not necessarily result in subsequent hepatitis, and the conditions that lead to migration of the organism from the intestine to the liver have not been determined. *H. hepaticus* appears to reside primarily within the bile canaliculi. Male mice were reported to have a greater incidence and severity of hepatitis than female mice, and this finding occurred in NTP studies as well. The recently identified *H. bilis*, like *H. hepaticus*, colonizes the biliary tract, liver, and intestine of mice. While *H. bilis* has been identified in animals with chronic hepatitis, whether it caused the hepatitis is not known (Fox *et al.*, 1995).

The pathogenesis of *H. hepaticus*-induced disease has not been fully characterized. In susceptible strains of mice, *H. hepaticus* can cause acute, focal, nonsuppurative, necrotizing hepatitis, which progresses to chronic, active hepatitis characterized by minimal necrosis, hepatocytomegaly, oval cell hyperplasia, and

cholangitis. *H. hepaticus* has been found to possess high levels of urease (Fox *et al.*, 1994). *H. hepaticus* is often isolated from the cecum and colon but is not necessarily isolated from the liver of A/JCr mice, even though these animals develop severe hepatitis. Culture supernatants from several strains of *H. hepaticus* and several other *Helicobacter* species were shown to cause cytopathic effects in a rodent hepatocyte cell line (Taylor *et al.*, 1995). Ward *et al.* (1996) suggested that autoimmunity may play a role in the progressive hepatitis and carcinogenesis in livers infected with *H. hepaticus*.

NTP Infectious Disease Surveillance

In 1993, during the histological evaluation of an NTP 2-year study, pathologists identified a constellation of liver lesions (hepatitis) in control and treated male mice that was consistent with what would later be described in mice infected with *H. hepaticus* (Ward *et al.*, 1993, 1994a; Fox *et al.*, 1994). Subsequently, pathology results from all mouse studies begun since 1984 (67 two-year studies) were reviewed for diagnoses of the characteristic hepatitis; the lesions were identified in nine studies (NTP, 1998a,b,c,d,e,f). Silver stains revealed helical bacteria consistent with *Helicobacter* present in the liver of male mice in the nine studies.

Every reasonable measure is taken to prevent the occurrence of infectious diseases during NTP 2-year carcinogenicity studies. When infections occasionally occur, care is taken to identify the causal agent and its source, measures are taken to ensure that animals in later studies will not be infected, and the potential impact on biological parameters (primarily neoplastic endpoints) important in interpretation of the study is determined. To date, animals (control and treated) from a few studies have had a mild pulmonary inflammatory response presumed to be caused by an infectious agent. In other studies, there have been utero-ovarian infections with *Klebsiella* sp. (Rao *et al.*, 1987) and fungal infections of the nasal cavity. For scientifically valid reasons, interpretation of chemical-related effects was not considered significantly compromised in any of these studies. Unlike the previous infections, *H. hepaticus* involves the liver, the major metabolic organ, and has been associated with an increase in incidences of liver neoplasms in the A/JCr mouse (Ward *et al.*, 1994a). Therefore, when the contemporary epizootic of *H. hepaticus* infection in the United States affected several NTP studies, use of the data for hazard identification was questioned. The first step was to determine the extent of the infection within NTP studies and then evaluate the impact the infection had on biological parameters important in interpretation of the carcinogenic potential of test chemicals.

MATERIALS AND METHODS

Histologic Examination

Studies in which mice were potentially infected with *H. hepaticus* were identified by reviewing the summary pathology tables for characteristic diagnoses: oval and/or biliary epithelial hyperplasia, hepatocyte enlargement (often diagnosed as karyomegaly), chronic inflammation, and regenerative hyperplasia. All 13-week and 2-year studies begun by the NTP since 1984 and for which complete pathology data were available (67 two-year studies) were examined. Eight contemporary studies in which the characteristic lesions were not identified from pathology tables were randomly selected for histologic reevaluation. Slides containing sections of hematoxylin- and eosin-stained livers from 20 to 25 control and 20 to 25 high-dose male mice from each of seven 2-year studies and one 13-week study (10 animals from each group) were reexamined microscopically for the presence of hepatitis potentially related to *H. hepaticus* infection. Hepatitis consistent with that observed with *H. hepaticus* infection was not observed in any of these studies.

Liver sections from five or more animals from each of nine 2-year studies in which hepatitis was observed were prepared using the Warthin-Starry silver stain or Steiner's modification to identify silver-positive helical bacteria.

PCR-RFLP Detection of *Helicobacter* DNA

Assays based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) were conducted at the NIEHS (Malarkey *et al.*, 1997) and the University of Missouri Research Animal Diagnostic and Investigative Laboratory (MU-RADIL) (Riley *et al.*, 1996) on liver tissue from approximately 20 animals from each of 32 NTP 2-year studies (including the nine affected studies) and three NTP 13-week studies. The majority of these studies were selected because they were begun at approximately the same time (1988-1990) as the nine affected studies. Also, two earlier studies (1984-1985; mouse life-span and *p*-nitroaniline studies) and one later study (1993; methyleugenol) were selected. The mouse life-span study was designed to evaluate the incidences of spontaneous changes associated with age; therefore, there is no NTP Technical Report. Pathology peer review is not complete for the methyleugenol study, and the NTP Technical Report (NTP, 1998g) has not been completed. Frozen tissue was available from 22 of these studies, while only formalin-fixed tissue was available for the remaining ten 2-year studies and the three 13-week studies. Most of the assays were conducted by MU-RADIL, which used *Helicobacter* genus-specific primers; MU-RADIL used restriction endonucleases on a subset of positives to determine if the species was *H. hepaticus*. DNA was isolated from frozen liver samples with a QIAamp Tissue Kit (Qiagen Inc., Chatsworth, CA) according to the manufacturer's recommendations or routine phenol/chloroform extraction (Malarkey *et al.*, 1997). DNA content and purity were determined spectrophotometrically by measuring the A_{260}/A_{280} optical density ratio. To isolate DNA from paraffin-embedded samples, five 10-µm sections were washed twice with 1 mL xylene and twice with 500 µL ethanol. Tissues were then dried within a vacuum centrifuge prior to DNA isolation as described above. Routine measures were taken to avoid contamination at every step from tissue collection to PCR amplification, and concurrently run controls without DNA were consistently negative.

Statistical Analyses

Multiple regression procedures were used to compare control neoplasm rates in the nine affected studies with the 26 unaffected contemporary studies which had no histologic evidence of *H. hepaticus*-associated liver disease. While frozen liver tissue was unavailable from 13 of these 26 studies, none showed the hepatitis indicative of *H. hepaticus* and thus were assumed to be unaffected. Potential confounding factors such as body weight, date study was begun, route of administration, and animal supplier were included as covariables in the statistical analysis.

Analysis for H-*ras* Codon 61 CAA-to-AAA Mutations

For analyses of formalin-fixed tissue, three to five unstained serial sections (10 µm thick) were cut from paraffin blocks containing hepatocellular adenomas or carcinomas. Paraffin-embedded tissues were deparaffinized and rehydrated prior to being digested with proteinase k overnight at 55° C to isolate DNA. Frozen tissues were digested with 10 mg/mL pronase in 1% sodium dodecyl sulfate in TNE buffer (10 mM TRIS, 150 mM NaCl, and 2 mM EDTA; pH 7.5) overnight at 37° C; DNA was isolated by phenol chloroform extraction and precipitated with ethanol (Marmur, 1961; Sills *et al.*, 1995).

Nested primers were used for amplification of exon 2 of H-*ras* by PCR. The outer primers were 5'-CCA CTA AGC CTG TTG TGT TTT GCA G-3' (forward primer) and 5'-CTG TAC TGA TGG ATG TCC TCG AAG GA-3' (reverse primer). The inner primers (second round of amplification) were 5'-GAC ATC TTA GAC ACA GCA GTT-3' (forward primer) and 5'-GGT GTT GTT GAT GGC AAA TAC-3' (reverse primer). Although the normal sequence of codon 60 is GCT, the forward PCR primer is made with a T at the penultimate 3' base to create the restriction site for Mse1.

A nonradioactive RFLP method was employed to identify CAA-to-AAA mutations in the H-*ras* gene at codon 61 in liver neoplasms (Lee and Drinkwater, 1995). This was based on Mse1 enzyme restriction cutting only the sequence 5'-TTAA-3'. Thus, Mse1 will detect C→A conversion mutation at the first position of codon 61.

Analysis of PCNA and Apoptosis

Detailed methods are included in a report by Nyska *et al*. (1997). Cell proliferation was assessed in nonneoplastic areas of the liver, kidney, and lung by determining a PCNA S-phase labeling index (the percentage of cells in S phase). The identification of apoptotic cells was based on morphologic criteria (Garewal *et al.*, 1996; Goldsworthy *et al.*, 1996) and confirmed immunohistochemically by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) procedure (Gavrieli *et al.*, 1992).

RESULTS AND DISCUSSION

Identification of *H. hepaticus* Infection in NTP Studies

Determining the extent of *H. hepaticus* infection involved a three-pronged approach of histologic evaluation, silver stains, and PCR-RFLP based assays; all were necessary because of the limitations identified for each. In NTP studies, and as reported in other studies (Ward *et al.*, 1994b), there were no obvious clinical signs of infection, and the only significant histologic lesion (hepatitis) was observed in the liver, primarily in males. Therefore, summary pathology tables were reviewed to identify studies that may have been affected by *H. hepaticus*-associated hepatitis. Male mice from nine studies were identified (Table L1) as having the hepatitis. Eight of the nine studies were begun during a time span of about 6 months (July 1990 to January 1991), while the other study was begun much earlier (October 1988). The hepatitis was not observed in any 13-week studies. Use of histologic evaluation for identification of infected animals has limitations, however. It is somewhat insensitive, as *H. hepaticus* has been cultured and identified by PCR-RFLP methods within livers of animals with no histological evidence of infection (Fox *et al.*, 1998). This may be explained in part by the limited sampling (two liver sections) and the sometimes focal nature of *H. hepaticus*-associated hepatitis. Also, while in the more severely affected animals the hepatitis appears somewhat characteristic, component lesions of the hepatitis are not pathognomonic, and, when the hepatitis is subtle in 2-year old animals, it is more difficult to recognize or attribute to *H. hepaticus*.

Within affected studies, the incidences of the hepatitis in male mice varied from 16% to 78% (Table L1). While generally mild to moderate, the hepatitis varied in severity from barely detectable in some animals to extensive liver involvement and regeneration in others. Only a few females were identified as having the characteristic hepatitis (Table L1). In general, the incidences and severities of *H. hepaticus*-associated hepatitis were similar between control and treated groups. This constellation of nonneoplastic liver lesions, while not pathognomonic, was certainly suggestive of an *H. hepaticus* infection, particularly when observed in control animals. Characteristic lesions included proliferation of oval and/or biliary epithelial cells, hepatocyte enlargement (diagnosed as karyomegaly), and chronic inflammation. In many instances, areas of regenerative hyperplasia were identified within diseased liver.

Helicobacter spp. are not usually observed on routine histologic examination of hematoxylin and eosin-stained sections of liver. The methods for confirmation of infection with *Helicobacter* include Warthin-Starry silver stain or Steiner's modification (Garvey *et al.*, 1985) of this stain for direct microscopic observation of the organisms in tissue; however, this can be a relatively insensitive technique when few organisms are present. In most instances, histologic differentiation between *Helicobacter* species is not possible. Speciation can usually be accomplished with electron microscopy, but this technique is both time consuming and labor intensive. Microbiologic culture of feces, cecal smears, and fresh or frozen liver is also possible. Currently, assays involving amplification of the DNA of the organism using PCR are the most rapid and perhaps the most sensitive methods of detection, and the use of restriction endonucleases has allowed a determination of the species present. PCR-based methods also can be used on feces, cecal contents, or liver homogenates and are most sensitive when using fresh or frozen tissue (Riley *et al.*, 1996; Malarkey *et al.*, 1997).

Using Warthin-Starry silver stains or Steiner's modification on the livers of five or more animals per study, helical bacteria (*Helicobacter*) were identified in animals from the nine affected studies. In some animals, helical bacteria were numerous, suggesting a heavy bacterial burden in these infected animals. However, even in these animals with abundant organisms, few to none were observed in proliferative hepatic lesions such as foci and neoplasms. Helical bacteria were not identified in approximately 25% of males with moderate hepatitis and were rarely identified in males without hepatitis or in females. The absence of identification of helical organisms by silver stains does not preclude infection, nor does the presence of organisms confirm *H. hepaticus*. Based upon current knowledge, however, the characteristic liver lesions in $B6C3F_1$ mice, coupled with the presence of silver-positive helical organisms, are highly suggestive of *H. hepaticus* infection.

As the NTP evaluation evolved, PCR-based assays were developed that appeared more sensitive than histologic evaluation and silver stains for identification and speciation of *Helicobacter*. Therefore, PCR-RFLP-based assays were used to confirm the presence of pathogenic *Helicobacter* (primarily *H. hepaticus*) within the nine affected studies and to determine whether there was *H. hepaticus* infection in other NTP studies. Unfortunately, none of the PCR-based assays had been specifically developed for, or proven reliable for use with, formalin-fixed tissue. Frozen tissue was available from a limited number of animals from a limited number of NTP studies, including only three of the nine affected studies. Furthermore, available frozen liver was almost always limited to tissue from a neoplasm, and, based upon results obtained with silver stains, organisms are generally not readily observed within proliferative hepatic lesions, even when organisms are abundant in adjacent liver tissue. Because the availability of frozen tissue was limited, a PCR-RFLP-based assay was developed and evaluated (Malarkey *et al.*, 1997) for use with frozen or formalin-fixed tissue.

The NIEHS and MU-RADIL laboratories conducted PCR-RFLP-based assays on 32 NTP 2-year studies and three NTP 13-week studies (data not shown); frozen tissues from 22 of the 2-year studies were available. All three bioassays in which hepatitis was identified and for which frozen tissue was available were positive for *H. hepaticus* by the PCR-RFLP-based assays (Table L2). At a third laboratory, *H. hepaticus* was also cultured from the liver tissue of animals in one of these studies (Fox *et al.*, 1998). Formalin-fixed tissues from two of the three studies were evaluated and were also positive; these tissues had been fixed in formalin for less than 48 hours. In the other six affected studies, for which only formalin-fixed tissue was available, *H. hepaticus* was identified in only 1 of 120 animals (Table L2). This decreased sensitivity was considered to be related to the prolonged formalin fixation (Malarkey *et al.*, 1997) rather than proof of an absence of *H. hepaticus*. The presence or absence of *H. hepaticus* apparently cannot be confirmed with current PCR-RFLP-based assays in liver that has been fixed in formalin for long periods (weeks or months). In the three 13-week studies with formalin-fixed tissue, only 1 of 30 animals was positive for *H. hepaticus*.

Within the three affected, PCR-RFLP-positive 2-year studies, *H. hepaticus* was often identified by PCR in frozen livers of mice that had no overt hepatitis. In fact, based upon the combined data from two studies (including PCR results from three laboratories), of 57 animals without characteristic liver lesions, 13 of 24 male mice (54%) and 17 of 33 female mice (52%) were positive for *H. hepaticus*. Furthermore, *H. hepaticus* was identified by PCR in frozen liver of several animals from three "unaffected" studies in which hepatitis typical of that associated with *H. hepaticus* was not observed (Table L2). Apparent variability occurs between various strains of mice and between individual mice from affected studies in developing hepatitis in response to *H. hepaticus* infection. One would assume that, within affected studies, most or all animals have been exposed to the organism, and even animals resistant to developing hepatitis may have organisms within the liver. This assumption is supported by the fact that animals without hepatitis are often positive with PCR-RFLP-based assays. Therefore, although alternative explanations are possible, the three PCR-RFLP-positive studies in which liver lesions are absent are assumed to be true positives. In fact, helical organisms were identified with a silver stain in one animal from one of these studies (Malarkey *et al.*, 1997). Therefore, in addition to assessing the affect of *H. hepaticus* in the nine affected 2-year

studies, the significance of a positive PCR-RFLP assay for *H. hepaticus* in the absence of liver lesions is also an important question.

Inconsistent Results with PCR-Based Methods

As with any technique, the PCR-RFLP-based assays have limitations even when used to assay fresh and frozen tissue. One assessment of the variability in results of PCR and serologic analyses for *Helicobacter* among three commercial laboratories revealed significant inconsistencies (Dew *et al.*, 1997). Others (J.M. Ward and J. Thigpen, personal communications) have obtained similarly inconsistent results when sending replicate samples to different laboratories. Though the number of samples evaluated by both the NIEHS and MU-RADIL laboratories was limited, there was good, but not complete, correlation of PCR-RFLP results. Also, within the affected studies, the PCR assays were not positive in some animals with liver disease. This result may be explained, in part, by the fact that the only frozen tissues available were neoplasms; as described above, neoplasms are expected to have fewer organisms.

Analysis of *H. hepaticus*-Affected and Unaffected Studies for Incidence of Common Neoplasms

To determine whether the incidences of various neoplasms were different between control groups from affected and unaffected studies, the nine affected studies were compared to 26 unaffected studies begun at relatively similar times (Table L3). There were no statistically significant differences in body weight or survival among the affected and unaffected studies. The neoplasms evaluated represent those that occurred at high enough incidences in various organs for statistically significant differences to be detected. Using multiple regression procedures, male mice in the nine affected studies were demonstrated to have a significantly ($P < 0.05$) increased incidence of only two neoplasm types, both of which were in the liver (hepatocellular neoplasms and hemangiosarcoma), when compared to the unaffected studies. Because of these differences, there was also a corresponding significant difference in the overall incidence of malignant neoplasms (all sites) as well as in the overall proportion of neoplasm-bearing animals. No other tissue site showed a significant difference in the incidence of neoplasms. For female mice, the slightly increased incidence of hepatocellular neoplasms observed in the affected studies was not statistically significant.

This seemingly simple analysis is complicated by several potential confounding variables. There have been coordinate, time-related increases in body weight and in the incidence of liver neoplasms in mice in NTP studies (Haseman, 1992). Table L4 presents the liver neoplasm incidences in relation to the dates the studies began and clearly shows the increases in liver neoplasm incidences and body weights (Seilkop, 1995). In assessing differences in neoplasm incidences between *H. hepaticus*-affected and unaffected studies, the most relevant comparison would be between studies begun at approximately the same time. The starts of 20 of the 26 unaffected studies were clustered near the early part of the time frame (April 1988 to June 1990), while the starts of the affected studies were clustered toward the later end, with eight of the nine studies begun between July 1990 and January 1991; incidences of liver neoplasms in these later studies are expected to be higher based on trends in body weight alone. While the slightly increased incidences of liver neoplasms observed in female control mice in the nine affected studies is likely due to clustering in time, clearly, this alone cannot account for the increased liver neoplasm incidences observed in control male mice in the affected studies (Table L3).

Ideally, unaffected studies used in the above comparison should not only be free of histologic evidence of infection with *H. hepaticus* but should be confirmed as negative by PCR assays. Thirteen of these 26 studies could not be confirmed as negative by PCR because frozen tissue was not available; however, *H. hepaticus*-associated hepatitis was not present in any of the 26 studies. Because these and other data reported to date suggest that hepatitis is associated with neoplasm development in the liver, it seems reasonable to include those 13 studies, unconfirmed by PCR, in this analysis. The majority of the 13 studies confirmed as negative by PCR were begun much earlier than the clearly affected studies, and, therefore, comparing them alone to the nine affected studies is not reasonable. Although not presented here, a number

of comparisons were made with various groupings of studies based on the degree of confidence in their infection status. Although the outcomes of the various comparisons varied somewhat, incidences of hepatocellular neoplasms and hemangiosarcomas of the liver were consistently increased in control male mice from affected studies compared to control males from unaffected studies. Significantly increased liver neoplasm incidences generally were not observed in females. Importantly, the following data corroborate the findings and association with *H. hepaticus* identified in these analyses.

Analysis of Hepatitis-Positive and Hepatitis-Negative Mice for Liver Neoplasm Incidence

Several infectious agents known to be associated with increased incidences of neoplasms cause chronic inflammation in the target tissue or organ. It is commonly hypothesized that this inflammatory process may cause or contribute to the development of neoplasms. One approach to address this was to stratify the mice from the affected studies according to the severity of hepatitis and examine liver neoplasm incidences in relation to these groupings. Thus, animals within the nine affected studies were placed into three groups: 1) animals with mild to moderate hepatitis considered related to *H. hepaticus* infection (+), 2) animals with minimal to mild hepatitis that may have been associated with *H. hepaticus* (±), and 3) animals with no hepatitis that was considered to be associated with *H. hepaticus* (–). Within these groupings, the incidence of liver neoplasms was significantly increased ($P < 0.05$) in males with mild to moderate *H. hepaticus*-associated hepatitis (+) when compared to animals without such hepatitis (Table L5). The neoplasm incidence in animals with minimal lesions (±) was also increased. The liver neoplasm incidence in males without hepatitis (58%) was similar to the incidence (54.8%) in males from the 26 unaffected studies (Table L3). This analysis clearly suggests an association of *H. hepaticus*-associated hepatitis with increased liver neoplasm incidences. Females showed a similar trend, albeit not significant; however, these comparisons are weak because of the low numbers of females with hepatitis.

Analysis of H-*ras* Oncogene Mutations in Liver Neoplasms in Mice from Affected and Unaffected Studies

Liver neoplasms commonly occur in control $B6C3F_1$ mice in 2-year studies. In the historical database of 333 male and female mice with liver neoplasms, 106 (32%) had H-*ras* codon 61 CAA-to-AAA mutations (Maronpot *et al.*, 1995). This historical control database is composed primarily of male data; however, adequate numbers of females have been assayed, and there was no significant difference in the incidences of CAA-to-AAA mutations between males and females.

In an attempt to examine further whether *H. hepaticus* infection had an effect on the development of hepatocellular neoplasms, neoplasms from control male mice from selected affected (NTP, 1998a,b,c) and unaffected (NTP, 1993, 1998h) studies were evaluated for H-*ras* codon 61 CAA-to-AAA mutations (Table L6). Only 6% (2/33) of the hepatocellular neoplasms from control males with hepatitis from three affected studies had this mutation. This percentage is significantly ($P < 0.01$) less than the 32% (11/34) observed in males from the two unaffected studies and less than the 32% (106/333) that occurred in historical control animals. In addition, neoplasms from males without hepatitis from the affected, PCR-positive triethanolamine study (NTP, 1998a) and the unaffected, PCR-positive methyleugenol study (NTP, 1998g) were evaluated; the incidences of mutations in those groups were 3/14 (21%) and 2/17 (12%), respectively.

Neoplasms from control female mice (none had hepatitis) from affected and unaffected studies were evaluated for the CAA-to-AAA mutation (Table L6). The mutation rate was low in both the affected studies (1/25; 4%) and the unaffected study (1/11; 9%) when compared to the 32% observed in the historical control groups.

The finding of a different H-*ras* mutation profile in neoplasms of male mice from affected studies tends to support the association of increased neoplasm incidences with *H. hepaticus*, although there is no mechanistic

understanding behind this observation. In a study of *H. hepaticus*-infected A/JCr mice, *ras* mutations were not detected in the 25 hepatocellular neoplasms analyzed using a PCR/single-strand conformation polymorphism assay (Sipowicz *et al.*, 1997). Because of the low spontaneous rate of liver neoplasms in the A/JCr mouse, there are few or no conclusive data on *ras* mutations in uninfected animals, however. Point mutations at codons 12, 13, and 61 of the Ki-, Ha- and N-*ras* genes were not identified in 45 early gastric carcinomas in humans, whether or not *H. pylori* was present (Craanen *et al.*, 1995). If the increased incidence of hepatocellular neoplasms is associated with hepatitis, as many suspect, then one would expect the neoplasms from animals without hepatitis to have a similar mutational profile as that of the historical controls. The data do not provide a clear answer, because the hepatitis-free males from the affected triethanolamine study (NTP, 1998a) and the males from the methyleugenol study (NTP, 1998g), which were positive by PCR but lacked hepatitis, had mutation frequencies between those of the unaffected controls and the hepatitis-positive mice. Furthermore, mutations in neoplasms from females, none of which had hepatitis, from two affected and one unaffected study were very low compared to the historical controls. These findings were unexpected, and their significance is not understood.

H. hepaticus-Associated Alterations in Cell Kinetics

Studies evaluating cell kinetics were completed to explore further the link between hepatitis and the increased incidence of liver neoplasms (Table L7; Nyska *et al.*, 1997). One of the major objectives was to determine whether there were differences between PCNA labeling indices in the livers of animals with hepatitis from three affected studies, cobalt sulfate heptahydrate, chloroprene, and triethanolamine (NTP, 1998a,b,c), compared to animals without hepatitis, whether from the same three affected studies or from an unaffected study, 1-trans-delta^9-tetrahydrocannabinol (NTP, 1996). Male mice with hepatitis from the three affected studies had a significantly increased ($P < 0.001$) labeling index, with a 24-fold increase over males from the unaffected study and a sixfold increase over males without hepatitis from the same three affected studies (Table L7). The labeling index increase in these mice was substantial and was considered biologically significant. Male mice without hepatitis from the three affected studies had a significantly greater labeling index (increased fourfold) than male mice from the unaffected study (Table L7). The significance of this finding is uncertain, as differences of a similar magnitude were observed in other comparisons. For example, the labeling index of females from the unaffected 1-trans-delta^9-tetrahydrocannabinol study (Table L7; NTP, 1996) was increased fivefold over females from the PCR-positive, hepatitis-negative scopolamine hydrobromide trihydrate study (NTP, 1997). Such differences may be within the limits of normal variability for 2-year-old animals.

A second objective of the cell proliferation studies of the liver was to determine if labeling indices were increased in animals from the PCR-positive, hepatitis-negative methyleugenol (NTP, 1998g), scopolamine hydrobromide trihydrate (NTP, 1997), and mouse life-span studies compared to an unaffected PCR-negative and hepatitis-negative 1-trans-delta^9-tetrahydrocannabinol study (NTP, 1996). The scopolamine hydrobromide trihydrate study was evaluated and included in the study by Nyska *et al.* (1997), while the methyleugenol and mouse life-span studies were completed later and are included in Table L7. The labeling indices of males from two of these three studies were almost identical to those of males from the unaffected study. However, the labeling index of males from the mouse life-span study is increased approximately fivefold over that of males from the unaffected study as well as fivefold over the labeling indices of males from the two like studies of scopolamine hydrobromide trihydrate and methyleugenol. This finding suggests that the increase observed in the mouse life-span study is not attributable to the presence of *H. hepaticus*, as two other studies also positive for *H. hepaticus* did not show a similar increase.

The cell proliferation data for the liver from NTP studies are consistent with data from a study by Fox *et al.* (1996) in which cell proliferation indices were evaluated at 8, 10, and 13 months in the A/JCr mouse, which is generally believed to be more susceptible to *H. hepaticus*-associated hepatitis than the B6C3F$_1$ mouse. In the study by Fox *et al.* (1996), cell proliferation rates were significantly increased at all time points in males. Some increases were observed in females in that study but did not reach statistical significance. An increased

incidence of hepatocellular neoplasms was observed only in the males. Though liver lesions were observed in females in that study, they were less severe than those in males.

In addition to the liver, cell proliferation indices (PCNA) were evaluated in the kidneys and lungs of male and female mice in affected studies versus those in unaffected studies (Nyska *et al.*, 1997). No apparent effect of *H. hepaticus* infection or the presence of hepatitis on PCNA indices was observed for the kidneys or lungs.

Apoptosis (programmed cell death) is another important parameter in evaluations of cell kinetics. The apoptotic index in the liver of male mice with hepatitis from an affected study, cobalt sulfate heptahydrate (NTP, 1998b), was significantly ($P < 0.01$) greater than that observed in males from the unaffected 1-trans-delta^9-tetrahydrocannabinol study and the PCR-positive, hepatitis-negative scopolamine hydrobromide trihydrate study (Nyska *et al.*, 1997). For females, there were no significant differences among the three studies.

Two 13-week studies which were begun during the same time as the nine affected studies were randomly selected for evaluation of PCNA indices. *H. hepaticus* was not identified in either of the studies by PCR-RFLP; however, as with all NTP 13-week studies, only tissue fixed in formalin for an unspecified period was available. Because of this, no true negative control group was available; therefore, the labeling index of these 19- to 20-week-old animals was compared to values cited in the literature (Eldridge and Goldsworthy, 1996) for 20-week-old B6C3F$_1$ mice. The labeling index in the NTP studies clearly was not increased (data not shown).

The Impact of *H. hepaticus* on the Interpretation of 2-Year Carcinogenesis Studies

Increases in the incidences of neoplasms are associated with a number of infectious agents. The chronic inflammation caused by these agents has been hypothesized to be important in the pathogenesis of the increased neoplasm incidences (e.g., gastric cancer associated with *H. pylori*). The increased incidences of liver neoplasms in male mice from the nine affected NTP studies were observed in the animals with *H. hepaticus*-associated hepatitis. Neoplasms from males with hepatitis tended to have an H-*ras* mutation profile different from that of animals from unaffected studies. Further, cell replication rates at 2 years were significantly higher in males with hepatitis compared to those in males without hepatitis. The data suggest that *H. hepaticus*-associated hepatitis is associated with the increased incidences of liver neoplasms in the male B6C3F$_1$ mouse. Therefore, the most important consideration in evaluating the impact of *H. hepaticus* infection on the interpretation of study results appears to be the presence or absence of significant hepatitis.

For any carcinogenicity study, data within and specific to the individual study provide the greatest basis for an accurate interpretation. However, it is prudent to consider and evaluate all data or information which may affect the interpretation. Based upon the data presented in this and other reports, general guidelines emerge that may be useful in interpreting potential chemical-associated carcinogenic effects in *H. hepaticus*-infected B6C3F$_1$ mice. In a study with sufficient evidence of *H. hepaticus*-associated hepatitis (> 10% of the animals having the characteristic hepatitis may be a reasonable guideline), interpretation of increased incidences of liver neoplasms (hepatocellular neoplasms and hemangiosarcoma) of male mice is considered to be potentially confounded.

Altered chemical uptake and metabolism, due to the intestinal load of *H. hepaticus* and to *H. hepaticus*-associated liver disease, respectively, are possible reasons for considering that the male mouse response to chemical administration at sites other than the liver should also be considered confounded. Data do not currently exist that definitively answer this question. In this group of nine studies, however, there is no evidence to suggest that affected mice responded to chemical treatment in organs other than the liver in a manner different from mice in nonaffected studies. Within each study, there was excellent concordance in chemical-associated neoplasms between the male mice and the females, which had little or no hepatitis

(Table L8). Furthermore, analyses indicate that *H. hepaticus* is not associated with neoplastic responses outside the liver; incidences of neoplasms at sites other than the liver were not different between control groups from affected and unaffected studies (Table L3). Cell replication rates in two major organs (lung and kidney) also were not increased in control groups from affected studies compared to those from unaffected studies.

One of the more difficult issues to address is whether interpretation of a treatment-related increase in liver neoplasm incidences in the female mouse is confounded when *H. hepaticus*-associated hepatitis is present within the male mice in the study. Most evidence to date links hepatitis with the increased liver neoplasm incidences observed in males, and female B6C3F$_1$ mice in affected studies do not have significant hepatitis at 2 years. The lack of hepatitis in females, however, is based on an analysis in which only late time points were evaluated histologically. Therefore, it is conceivable that hepatitis along with increased cell proliferation could have occurred earlier and resolved by 18 months to 2 years. Data collected to date, however, suggest that *H. hepaticus*-associated hepatitis is a late-developing and persistent disease in the B6C3F$_1$ mouse. *H. hepaticus*-associated hepatitis has never been observed in any NTP 13-week studies, including five begun during the same 6-month time span as eight of the nine affected 2-year studies. Also, within affected 2-year studies, more males (51%) that were 18 to 24 months of age had hepatitis than those (34%) that were 12 to 18 months of age. This is consistent with a report by Ward *et al*. (1994b) that *H. hepaticus*-associated liver lesions are not observed at early time points in the B6C3F$_1$ mouse.

Nonetheless, within affected studies, female control mice did have a slightly elevated incidence of liver neoplasms when compared to control mice from unaffected studies, and the data derived from the H-*ras* mutation frequency analysis were inconclusive. The possibility that *H. hepaticus*-infected female mice from affected studies may respond differently to a liver carcinogen than mice from unaffected studies cannot be eliminated at this time. However, because within an affected study hepatitis is observed only rarely in females, until definitive data suggest otherwise, it is concluded that the interpretation of an apparent chemical-induced neoplastic effect in the liver of female mice is not confounded. To censor the few females with *H. hepaticus*-associated hepatitis from any statistical analyses of hepatocellular neoplasms would be prudent. Studies in the ostensibly more sensitive A/JCr mouse (Fox *et al.*, 1996) also showed significant increases in neoplasm incidences and cell proliferation rates in the liver of *H. hepaticus*-infected males, but not females.

Another concern is how to interpret possible chemical-related effects in a study in which the status of *H. hepaticus* infection cannot be determined by PCR-RFLP because only tissues fixed in formalin for more than 48 hours are available. While histologic evaluation is inadequate to identify infection, it appears adequate for identifying hepatitis severe enough to alter the outcome of the study. Therefore, in the absence of significant histologic evidence of *H. hepaticus*-associated hepatitis, the outcome of a 2-year study should not be considered potentially compromised.

The causality between *H. hepaticus* infection and neoplasia has not been proven in the B6C3F$_1$ mouse in these studies, nor has the mechanism of this association been determined; further studies are needed. However, sufficient information exists to make reasonable scientific judgments relative to the interpretation of data from the nine 2-year carcinogenicity studies in the B6C3F$_1$ mouse. Refinements to the above interpretive positions may occur if warranted by future information.

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TABLE L1
Incidence of *Helicobacter hepaticus*-Associated Hepatitis in Control B6C3F$_1$ Mice from Nine NTP 2-Year Studies[a]

Study	Incidence of Hepatitis (%)	
	Males	Females
Sodium xylenesulfonate	78	4
AZT/5,000 U α-interferon A/D	76	4
Cobalt sulfate heptahydrate	72	8
AZT/500 U α-interferon A/D	66	0
Chloroprene	54	0
Theophylline	32	0
α-Interferon A/D	22	4
Triethanolamine	20	0
AZT	16	2
Average	48	2

[a] Includes regeneration and mild to marked (excludes minimal) chronic inflammation, karyomegaly, oval cell hyperplasia, and bile duct hyperplasia. AZT=3′-azido-3′-deoxythymidine

TABLE L2
Identification of *Helicobacter hepaticus* with PCR-RFLP-Based Assays in Control B6C3F$_1$ Mice from 32 NTP 2-Year Studies and Three NTP 13-Week Studies[a]

Type of Sample	Total Studies	*H. hepaticus*-Positive Studies[b]	
		Affected Studies	Unaffected Studies
13-Week Studies			
Formalin-fixed liver	3	—	1/3[c]
2-Year Studies			
Frozen liver	22	3/3	3/19
Formalin-fixed liver	10	1/6[c]	0/4

[a] PCR-RFLP=polymerase chain reaction-restriction fragment length polymorphism
[b] Number of *H. hepaticus*-positive studies/number of affected or unaffected studies. Affected studies are those in which hepatitis typical of that associated with *H. hepaticus* infection occurred in many male mice.
[c] Only one animal in the positive study was positive for *H. hepaticus*.

TABLE L3
Comparison of Neoplasm Incidences in Control B6C3F$_1$ Mice from *Helicobacter hepaticus*-Affected and Unaffected NTP 2-Year Studies

	Males		Females	
	Affected Studies[a]	Unaffected Studies	Affected Studies	Unaffected Studies
Number of studies	9	26	9	26
Survival (%)	64	71	68	68
12-Month body wt (g)	48.0	48.3	48.1	47.0
Neoplasm incidence (%)				
Liver	71.3*	54.8	50.3	40.5
Lung	26.6	23.2	7.6	10.3
Pituitary gland	0.4	0.8	14.7	14.3
Harderian gland	5.6	6.1	6.0	4.9
Lymphoma	6.9	6.3	16.2	15.5
Circulatory system	9.8	6.0	5.3	4.7
liver only	7.1*	2.5	—	—
All benign	61.8	57.2	59.1	54.6
All malignant	61.3*	40.9	50.0	44.2
All neoplasms	88.0*	77.4	82.7	75.4

* Significantly different ($P \leq 0.05$) from the unaffected studies
[a] Affected studies are those in which hepatitis typical of that associated with *H. hepaticus* infection occurred in many male mice.

TABLE L4
Liver Neoplasm Incidences and Body Weights of Control B6C3F$_1$ Mice in Relation to Study Start Dates of *Helicobacter hepaticus*-Affected and Unaffected NTP 2-Year Studies[a]

Study Start Date	Liver Neoplasm Incidence (%)		Mean Body Weight (g)	
	Affected Studies[a]	Unaffected Studies	Affected Studies	Unaffected Studies
Male				
April to September 1988	—	43.8 (8)[b]	—	46.2 (8)
October 1988	62.0 (1)	—	48.3 (1)	—
November 1988 to September 1989	—	52.6 (7)	—	48.7 (7)
October 1989 to June 1990	—	61.2 (5)	—	48.9 (5)
July 1990 to January 1991	72.5 (8)	66.2 (4)	48.0 (8)	49.0 (4)
February 1991 to April 1992	—	68.0 (2)	—	52.8 (2)
Average	71.3	54.8	48.0	48.3
Female				
April to September 1988	—	31.1 (8)	—	44.8 (8)
October 1988	46.0 (1)	—	46.4 (1)	—
November 1988 to September 1989	—	39.9 (7)	—	47.2 (7)
October 1989 to June 1990	—	38.6 (5)	—	45.9 (5)
July 1990 to January 1991	50.9 (8)	54.2 (4)	48.3 (8)	48.0 (4)
February 1991 to April 1992	—	58.0 (2)	—	55.6 (2)
Average	50.3	40.5	48.1	47.0

[a] Includes nine affected studies (those in which hepatitis typical of that associated with *H. hepaticus* infection occurred in many male mice) and 26 unaffected studies
[b] Number of studies is given in parentheses.

TABLE L5
Association of Liver Neoplasm Incidence and Severity of *Helicobacter hepaticus*-Associated Hepatitis in Control B6C3F$_1$ Mice from Nine Affected NTP 2-Year Studies[a]

Severity of Hepatitis	Liver Neoplasm Incidence	
	Males	Females
Absent	101/175 (58%)	196/396 (49%)
Minimal	44/57 (77%)	23/42 (55%)
Mild/moderate	176/218 (81%)	7/11 (64%)
Significance of association	P < 0.05	NS[b]

[a] Affected studies are those in which hepatitis typical of that associated with *H. hepaticus* infection occurred in many male mice.
[b] NS=not significant

TABLE L6
H-*ras* Codon 61 AAA Mutations in Spontaneous Liver Neoplasms in Control B6C3F$_1$ Mice from *Helicobacter hepaticus*-Affected and Unaffected NTP 2-Year Studies

Study	Affected[a]	H-*ras* AAA Mutations
Male		
Cobalt sulfate heptahydrate	+	0/10 (0%)
Chloroprene	+	1/13 (8%)
Triethanolamine	+	1/10 (10%)
Oxazepam	—	7/18 (39%)
Diethanolamine	—	4/16 (25%)
Historical control database		106/333 (32%)
Female		
Chloroprene	+	0/10 (0%)
Triethanolamine	+	1/15 (7%)
Diethanolamine	—	1/11 (9%)
Historical control database		106/333 (32%)

[a] +=affected; —=not affected. Affected studies are those in which hepatitis typical of that associated with *H. hepaticus* infection occurred in many male mice.

TABLE L7
Proliferating Cell Nuclear Antigen Labeling Indices in the Liver of Control B6C3F$_1$ Mice[a]

	Hepatitis	No. of Animals	PCNA Labeling Index[b]	Average PCNA Labeling Index[c]
Male				
Cobalt sulfate heptahydrate[d]	+	15	0.535 ± 0.129	
Chloroprene[d]	+	12	1.452 ± 0.386	
Triethanolamine[d]	+	9	1.215 ± 0.374	1.011
Cobalt sulfate heptahydrate	—	7	0.175 ± 0.117	
Chloroprene	—	10	0.296 ± 0.124	
Triethanolamine	—	12	0.100 ± 0.042	0.186
1-Trans-delta^9-tetrahydrocannabinol[e]	—	15	0.042 ± 0.011	
Scopolamine hydrobromide trihydrate[f]	—	14	0.043 ± 0.012	
Methyleugenol[f]	—	14	0.077 ± 0.020	
Mouse life-span study[f]	—	15	0.217 ± 0.880	
Female				
Cobalt sulfate heptahydrate	+	5	0.161 ± 0.062	
Cobalt sulfate heptahydrate	—	17	0.055 ± 0.015	
Chloroprene	—	12	0.154 ± 0.050	
Triethanolamine	—	12	0.138 ± 0.053	0.108
1-Trans-delta^9-tetrahydrocannabinol	—	13	0.156 ± 0.047	
Scopolamine hydrobromide trihydrate	—	15	0.032 ± 0.009	

[a] A portion of these data are presented in Nyska *et al*. (1997). +=hepatitis present; —=no hepatitis present
[b] Mean ± standard error; PCNA=proliferating cell nuclear antigen
[c] Average of the mean labeling indices for animals from all three studies
[d] Affected study (one in which hepatitis typical of that associated with *H. hepaticus* occurred in many male mice)
[e] Unaffected study (one in which the typical hepatitis did not occur in mice)
[f] Unaffected study with no typical hepatitis, but positive for *H. hepaticus* by polymerase chain reaction-restriction fragment length polymorphism-based assay

TABLE L8
Summary of Target Sites of Carcinogenicity in B6C3F$_1$ Mice from NTP 2-Year Studies with *Helicobacter hepaticus*-Associated Hepatitis

	Males	Females
Chloroprene	Lung Circulatory system[a] Harderian gland Forestomach Kidney	Lung Circulatory system Harderian gland Forestomach Liver Skin Mesentery Zymbal's gland Mammary gland
Cobalt sulfate heptahydrate[b]	Lung	Lung
Triethanolamine	Liver	Liver
AZT[c]	None	Vagina
Sodium xylenesulfonate	None	None
Theophylline	None	None

[a] Hemangioma and hemangiosarcoma of the liver were excluded from the analysis in males.
[b] An apparent treatment-related increase in the incidence of hemangiosarcoma of the liver was discounted in male mice because of the presence of *H. hepaticus*.
[c] AZT=3′-azido-3′-deoxythymidine. Includes four studies: AZT; α-interferon A/D; AZT/500 U α-interferon A/D; and AZT/5,000 U α-interferon A/D